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(54) Title: ASSAYS FOR TASTE RECEPTOR CELL SPECIFIC ION CHANNEL

(57) Abstract: The invention includes nucleic acid and amino acid sequences of a taste-cell-specific ion channel subunit that is specifically expressed in taste cells, antibodies to such subunits, methods of detecting such nucleic acids and proteins, and methods of screening for modulators of taste cell specific ion channel subunits.

ASSAYS FOR TASTE RECEPTOR CELL SPECIFIC ION CHANNEL

CROSS-REFERENCES TO RELATED APPLICATIONS

The present application claims priority to USSN 60/259,379, filed December 29, 2000, herein incorporated by reference in its entirety.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This invention was made with Government support under Grant No. 5R01 DC03160, awarded by the National Institutes of Health. The Government has certain rights in this invention.

FIELD OF THE INVENTION

The invention identifies nucleic acid and amino acid sequences of an ion channel protein that is specifically expressed in taste cells, antibodies to the taste cell specific ion channel protein, methods of detecting such nucleic acids and subunits, and methods of screening for modulators of said taste cell specific ion channel.

BACKGROUND OF THE INVENTION

Taste transduction is one of the most sophisticated forms of chemotransduction exhibited by animals, from simple metazoans to the most complex of vertebrates. *See, e.g.*, Avenet & Lindemann, *J. Membrane Biol.* 112:1-8 (1989); Margolskee, *BioEssays* 15:645-650 (1993); Gilbertson, T. *The physiology of vertebrate taste reception* 3, 532-539 (1993); Kinnamon, S.C. and Margolskee, R.F. (1996), *Curr. Opin. Neurobiol.*, 4. 506-513; Roper, S.D. *Ann. Rev. Neurosci.* 12, 329-353 (1989); Hoon *et al.* (1999), *Cell* 96, 541-51; Adler *et al.* (2000), *Cell* 100:693-702; Chandrashekar *et al.* (2000), *Cell* 100:703-711. It enables animals to reliably detect and appropriately respond to chemical compounds present in their environment: for example, avoid toxic substances, foods, environments and enemies; or identify edible foods or sources of food, livable environments, and familiar or compatible individuals.

Higher organisms generally are able to discriminate between four basic types of taste modalities: salty, sour, sweet, and bitter. Mammals reportedly have five basic taste modalities: sweet, bitter, sour, salty and unami (the taste of monosodium glutamate) (*see, e.g.*, Kawamura & Kare, *Introduction to Unami: A Basic Taste* (1987); Kinnamon & Cummings, *Ann. Rev. Physiol.* 54:715-731(1992); Lindemann, *Physiol. Rev.* 76:718-766 (1996); Stewart *et al.*, *Am. J. Physiol.* 272:1-26 (1997)). Each of these modalities is thought to be mediated by distinct signaling pathways leading to receptor cell depolarization, generation of a receptor or action potential, and the release of neurotransmitter and synaptic activity (*see, e.g.*, Roper, *Ann. Rev. Neurosci.* 12:329-353 (1989)).

Extensive psychophysical studies in humans have reported that different regions of the tongue display different gustatory preferences (*see, e.g.*, Hoffmann, *Menchen. Arch. Path. Anat. Physiol.* 62:516-530 (1875); Bradley *et al.*, *Anatomical Record* 212: 246-249 (1985); Miller & Reedy, *Physiol. Behav.* 47:1213-1219 (1990)). Also, numerous physiological studies in animals have shown that taste receptor cells may selectively respond to different tastants (*see, e.g.*, Akabas *et al.*, *Science* 242:1047-1050 (1988); Gilbertson *et al.*, *J. Gen. Physiol.* 100:803-24 (1992); Bernhardt *et al.*, *J. Physiol.* 490:325-336 (1996); Cummings *et al.*, *J. Neurophysiol.* 75:1256-1263 (1996)).

In mammals, taste receptor cells are assembled into taste buds that are distributed into different papillae in the tongue epithelium. Each taste bud, depending on the species, contain 50-150 cells, including precursor cells, support cells, and taste receptor cells (*see, e.g.*, Lindemann, *Physiol. Rev.* 76:718-766 (1996)). Receptor cells are innervated at their base by afferent nerve endings that transmit information to the taste centers of the cortex through synapses in the brain stem and thalamus.

Circumvallate papillae, found at the very back of the tongue, contain hundreds (mice) to thousands (human) of taste buds and are particularly sensitive to bitter substances. Foliate papillae, localized to the posterior lateral edge of the tongue, contain dozens to hundreds of taste buds and are particularly sensitive to sour and bitter substances. Fungiform papillae containing a single or a few taste buds are at the front of the tongue and are thought to mediate much of the sweet taste modality.

Elucidating the mechanisms of taste cell signaling and information processing are critical for understanding the function, regulation, and "perception" of the sense of taste. Although much is known about the psychophysics and physiology of taste

cell function, very little is known about the molecules and pathways that mediate these sensory signaling responses (reviewed by Gilbertson, *Current Opn. in Neurobiol.* 3:532-539 (1993)). Electrophysiological studies suggest that sour and salty tastants modulate taste cell function by direct entry of H^+ and Na^+ ions through specialized membrane channels on the apical surface of the cell. In the case of sour compounds, taste cell depolarization is hypothesized to result from H^+ blockage of K^+ channels (*see, e.g.*, Kinnamon *et al.*, *PNAS USA* 85: 7023-7027 (1988)) or activation of pH-sensitive channels (*see, e.g.*, Gilbertson *et al.*, *J. Gen. Physiol.* 100:803-24 (1992)); salt transduction may be partly mediated by the entry of Na^+ via amiloride-sensitive Na^+ channels (*see, e.g.*, Heck *et al.*, *Science* 223:403-405 (1984); Brand *et al.*, *Brain Res.* 207-214 (1985); Avenet *et al.*, *Nature* 331:351-354 (1988)). Most of molecular components of the sour or salty pathways have not been identified.

Sweet, bitter, and unami transduction are believed to be mediated by G-protein-coupled receptor (GPCR) signaling pathways (*see, e.g.*, Striem *et al.*, *Biochem. J.* 260:121-126 (1989); Chaudhari *et al.*, *J. Neuros.* 16:3817-3826 (1996); Wong *et al.*, *Nature* 381:796-800 (1996)). Confusingly, there are almost as many models of signaling pathways for sweet and bitter transduction as there are effector enzymes for GPCR cascades (*e.g.*, G protein subunits, cGMP phosphodiesterase, phospholipase C, adenylate cyclase; *see, e.g.*, Kinnamon & Margolskee, *Curr. Opin. Neurobiol.* 6:506-513 (1996)). Identification of molecules involved in taste signaling is important given the numerous pharmacological and food industry applications for bitter antagonists, sweet agonists, and modulators of salty and sour taste.

The identification and isolation of taste receptors (including taste ion channels), and taste signaling molecules, such as G-protein subunits, ion channels and enzymes involved in signal transduction, would allow for the pharmacological and genetic modulation of taste transduction pathways. For example, availability of receptor, ion channels, and other molecules involved in taste transduction would permit the screening for high affinity agonists, antagonists, inverse agonists, and modulators of taste cell activity. Such taste modulating compounds could then be used in the pharmaceutical and food industries to customize taste. In addition, such taste cell specific molecules can serve as invaluable tools in the generation of taste topographic maps that elucidate the relationship between the taste cells of the tongue and taste sensory neurons leading to taste centers in the brain.

SUMMARY OF THE INVENTION

The present invention demonstrates, for the first time, taste cell-specific expression of nucleic acids encoding an ion channel subunit. The taste cell-specific ion channel subunits that are specifically expressed in taste cells can thus be used to screen for modulators of taste cell function and to control taste perception. The compounds identified by these assays would then be used by the food and pharmaceutical industries to customize taste, *e.g.*, as additives to food or medicine so that the food or medicine tastes different to the subject who ingests it. For example, bitter medicines can be made to taste less bitter, and sweet substance can be enhanced.

Using isolated, hand-dissected taste buds and papillae from the rat circumvallate papillae, subtracted cDNA libraries against non-taste lingual tissue were generated and screened for sequences preferentially expressed in taste receptor cells. Clones representing differentially expressed genes were isolated, mapped by *in situ* hybridization to single taste receptor cells, and used as probe to isolate and characterize full length cDNA sequences. This procedure led to the isolation of a novel taste-specific ion channel.

In one aspect, the present invention provides a method for identifying a compound that modulates transduction of taste signals in taste cells, the method comprising the steps of: (i) contacting the compound with a eukaryotic host cell or cell membrane in which has been expressed a taste cell-specific ion channel subunit having (a) greater than about 70% amino acid sequence identity to a polypeptide having a sequence of SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:8; and (b) specifically binding to polyclonal antibodies generated against SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:8; and (ii) determining a functional effect of the compound upon the cell or cell membrane expressing the taste cell-specific ion channel subunit.

In one embodiment, the functional effect is determined by measuring changes in intracellular cAMP, cGMP, IP_3, DAG, or Ca^{2+}.

In another embodiment, the functional effect is determined by measuring changes in the level of phosphorylation of taste cell specific proteins.

In another embodiment, the functional effect is determined by measuring changes in transcription levels of taste cell specific genes.

In another embodiment, the taste cell specific ion channel subunits are recombinant.

In other embodiments, the taste cell-specific ion channel subunit have an amino acid sequence of SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:8.

In another aspect, the present invention provides a method identifying a compound that modulates taste signaling in taste cells, the method comprising the steps of: (i) expressing a taste cell-specific ion channel subunit in an HEK 293 host cell, wherein the taste cell-specific ion channel subunit: (a) has greater than about 70% amino acid sequence identity to a polypeptide having a sequence of SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:8; and (b) specifically binds to polyclonal antibodies generated against SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:8; (ii) contacting the host cell with the compound that modulates taste signaling in taste cells; and (iii) determining changes in intracellular calcium levels in the host cell.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a picture of an *in situ* hybridization of a nucleic acid of the present invention to a tissue section, demonstrating that the nucleic acid is specific for taste cells.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

The present invention relates to nucleic acids that encode taste cell-specific ion channel subunits that are specifically expressed in taste cells. These nucleic acids and the polypeptides that they encode are referred to as "TC-ICS" for "taste cell specific ion channel subunit." These taste cell specific nucleic acids and polypeptides are components of the taste transduction pathway.

The invention provides methods of screening for modulators, *e.g.*, activators, inhibitors, stimulators, enhancers, agonists, and antagonists of TC-ICS. Such modulators of taste transduction are useful for pharmacological and genetic modulation of taste signaling pathways. These methods of screening are used to identify high affinity agonists and antagonists of taste cell activity. These modulatory compounds can then be used in the food and pharmaceutical industries to customize and/or regulate taste. The

modulatory compounds typically would be added to a food or medicine, thereby altering its taste to the subject who ingests it.

Thus, the invention provides assays for taste modulation, where TC-ICS acts as a direct or indirect reporter molecule for the effect of modulators on taste transduction. TC-ICS are used in assays, *e.g.*, to measure changes in ion concentration, membrane potential, current flow, ion flux, transcription, signal transduction, receptor-ligand interactions, second messenger concentrations, *in vitro*, *in vivo*, and *ex vivo*. In one embodiment, a TC-ICS is recombinantly expressed in cells, and modulation of taste transduction is assayed by measuring changes in Ca^{2+} levels (*see* Example II). Methods of assaying for modulators of taste transduction include oocyte or tissue culture cell expression of TC-ICS; transcriptional activation of TC-ICS; phosphorylation and dephosphorylation of TC-ICS; ligand binding assays; voltage, membrane potential and conductance changes; ion flux assays; changes in intracellular second messengers such as cAMP and inositol triphosphate; changes in intracellular calcium levels; and neurotransmitter release.

These nucleic acids and proteins also provide valuable probes for the identification of taste cells, as the nucleic acids are specifically expressed in taste cells. For example, probes for TC-ICS are used to identify subsets of taste cells such as foliate cells and circumvallate cells, or specific taste receptor cells, *e.g.*, sweet, sour, salty, and bitter. They also serve as tools for the generation of taste topographic maps that elucidate the relationship between the taste cells of the tongue and taste sensory neurons leading to taste centers in the brain. Furthermore, the nucleic acids and the proteins they encode are used as probes to dissect taste-induced behaviors.

Finally, the invention provides for methods of detecting TC-ICS nucleic acid and protein expression, allowing investigation of taste transduction regulation and specific identification of taste receptor cells. TC-ICSs also provide useful nucleic acid probes for paternity and forensic investigations. TC-ICSs are useful nucleic acid probes for identifying subpopulations of taste receptor cells such as foliate, fungiform, and circumvallate taste receptor cells. TC-ICS polypeptides can also be used to generate monoclonal and polyclonal antibodies useful for identifying taste receptor cells, *e.g.*, in immuno histochemical assays. Taste receptor cells can also be identified using techniques such as reverse transcription and amplification of mRNA, isolation of total RNA or poly A^+ RNA, northern blotting, dot blotting, *in situ* hybridization, RNase

protection, S1 digestion, probing high density oligonucleotide arrays, western blots, and the like.

Functionally, TC-ICS represents a subunit of an ion channel involved in taste transduction. Structurally, the nucleotide sequence of TC-ICS (including SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:7, and also any sequence that encodes SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8) encodes a polypeptide of approximately 1125 amino acids. Related TC-ICS genes from other species share at least about 70% amino acid identity over an amino acid region at least about 25 amino acids in length, preferably 50 to 100 amino acids in length. *In situ* hybridization demonstrates tissue and cell-type specificity in taste buds.

Specific regions of the TC-ICS nucleotide and amino acid sequences are used to identify polymorphic variants, interspecies homologs, and alleles of TC-ICS. Especially useful are unique subsequences of SEQ ID NO:1, SEQ ID NO:4 and SEQ ID NO:7 that are at least 20, preferably at least 30, more preferably at least 50, most preferably at least 100 nucleotides long and that have at least 90-95% sequence homology with a subsequence present in SEQ ID NO:1, SEQ ID NO:4 *and* SEQ ID NO:7. Also especially useful are unique subsequences of SEQ ID NO:3 and SEQ ID NO:6 that are at least 20, preferably at least 30, more preferably at least 50, most preferably at least 100 nucleotides long and that have at least 90-95% sequence homology with a subsequence present in SEQ ID NO:3 *and* SEQ ID NO:6. This identification are made *in vitro*, *e.g.*, under stringent hybridization conditions or with PCR and sequencing, or by using the sequence information in a computer system for comparison with other nucleotide or amino acid sequences. Typically, identification of polymorphic variants and alleles of TC-ICS is made by comparing an amino acid sequence of about 25 amino acids or more, preferably 50-100 amino acids. Amino acid identity of approximately at least 70% or above, preferably 80%, most preferably 90-95% or above typically demonstrates that a protein is a polymorphic variant, interspecies homolog, or allele of TC-ICS. Sequence comparisons are performed using any of the sequence comparison algorithms discussed below. Antibodies that bind specifically to TC-ICS or a conserved region thereof can also be used to identify alleles, interspecies homologs, and polymorphic variants.

Polymorphic variants, interspecies homologs, and alleles of TC-ICS are confirmed by examining taste cell specific expression of the putative TC-ICS polypeptide. Typically, TC-ICS having the amino acid sequence of SEQ ID NO:2, SEQ

ID NO:5, or SEQ ID NO:8 is used as a positive control, *e.g.*, in immunoassays using antibodies specifically directed against a protein having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8, in comparison to the putative TC-ICS protein to demonstrate the identification of a polymorphic variant or allele of TC-ICS. Alternatively, TC-ICS having the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7 is used as a positive control, *e.g.*, in *in situ* hybridization with SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7, in comparison to the putative TC-ICS nucleotide sequences to demonstrate the identification of a polymorphic variant or allele of TC-ICS.

TC-ICS nucleotide and amino acid sequence information may also be used to construct models of taste cell specific polypeptides in a computer system. These models are subsequently used to identify compounds that can activate or inhibit TC-ICS. Such compounds that modulate the activity of TC-ICS are used to investigate the role of TC-ICS in taste transduction or are used as therapeutics.

Identification of taste cell specific expression of TC-ICS provides a means for assaying for inhibitors and activators of taste cell activity. TC-ICS is useful for testing taste modulators using *in vivo* and *in vitro* expression that measure, *e.g.*, transcriptional activation of TC-ICS; ligand binding; phosphorylation and dephosphorylation; binding to G-proteins; G-protein activation; regulatory molecule binding; voltage, membrane potential and conductance changes; ion flux; intracellular second messengers such as cAMP and inositol triphosphate; intracellular calcium levels; and neurotransmitter release. Such activators and inhibitors identified using TC-ICS are used to further study taste transduction and to identify specific taste agonists and antagonists. Such activators and inhibitors are useful as pharmaceutical and food agents for customizing taste.

Methods of detecting TC-ICS nucleic acids and expression of TC-ICS are also useful for identifying taste cells and creating topological maps of the tongue and the relation of tongue taste receptor cells to taste sensory neurons in the brain. Furthermore, these nucleic acids are used to diagnose diseases related to taste by using assays such as northern blotting, dot blotting, *in situ* hybridization, RNase protection, and the like. Chromosome localization of the genes encoding human TC-ICS can also be used to identify diseases, mutations, and traits caused by and associated with TC-ICS.

Techniques, such as high density oligonucleotide arrays (GeneChip™), are used to screen for mutations, polymorphic variants, alleles and interspecies homologs of TC-ICS.

II. Definitions

As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

"Taste receptor cells" are neuroepithelial cells that are organized into groups to form taste buds of the tongue, *e.g.*, foliate, fungiform, and circumvallate cells (*see, e.g.*, Roper *et al.*, *Ann. Rev. Neurosci.* 12:329-353 (1989)).

"Taste cell specific" genes or proteins refer to those which are expressed exclusively, or preferentially, in the taste receptor cells but not in non-taste cells, or in subsets of Gustducin positive cells.

"Taste cell-specific ion channel subunit" or "TC-ICS" refers to a family of taste cell-specific ion channel subunits that are specifically expressed in taste receptor cells such as foliate, fungiform, and circumvallate cells. The family includes proteins having the amino acid sequences of, *e.g.*, SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8, and they are encoded by cDNAs having the sequences of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7 and by genomic sequences such as, for example, SEQ ID NO:3 or SEQ ID NO:6. Such taste cells can be identified because they express molecules such as Gustducin, a taste cell specific G-protein (McLaughin *et al.*, *Nature* 357:563-569 (1992)). Taste receptor cells can also be identified on the basis of morphology (*see, e.g.*, Roper, *supra*). TC-ICS nucleic acids encode a taste cell-specific ion channel subunit with the ability to form a functional ion channel.

The term TC-ICS therefore refers to polymorphic variants, alleles, mutants, and interspecies homologs that: (1) have about 70% amino acid sequence identity, preferably about 85-90% amino acid sequence identity to SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8 over a window of about 25 amino acids, preferably 50-100 amino acids; (2) bind to antibodies raised against an immunogen comprising an amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8 and conservatively modified variants thereof; or (3) specifically hybridize (with a size of at least about 500, preferably at least about 900 nucleotides) under stringent hybridization conditions to a nucleic acid having SEQ ID NOS: 1, 4, or 7, and conservatively modified variants thereof.

"TC-GPCR" refers to a G-protein coupled receptor that is specifically expressed in taste receptor cells such as foliate, fungiform, and circumvallate cells. Such taste cells can be identified because they express molecules such as Gustducin, a taste cell specific G-protein (McLaughin *et al., Nature* 357:563-569 (1992)). Taste receptor cells can also be identified on the basis of morphology (*see, e.g.*, Roper, *supra*). TC-GPCR generally have seven transmembrane regions that have "G-protein coupled receptor activity," *e.g.*, they bind to G-proteins in response to extracellular stimuli and promote production of second messengers such as IP_3, cAMP, and Ca^{2+} via stimulation of enzymes such as phospholipase C and adenylate cyclase (for a description of the structure and function of G-protein coupled receptors, *see, e.g.*, Fong, *supra*, and Baldwin, *supra*).

A "host cell" is a naturally occurring cell or a transformed cell that contains an expression vector and supports the replication or expression of the expression vector. Host cells may be cultured cells, explants, cells *in vivo*, and the like. Host cells may be prokaryotic cells such as *E. coli*, or eukaryotic cells such as yeast, insect, amphibian, or mammalian cells such as CHO, HeLa, HEK 293 and the like.

"Biological sample" as used herein is a sample of biological tissue or fluid that contains nucleic acids or polypeptides of TC-ICS. Such samples include, but are not limited to, tissue isolated from humans, mice, and rats, in particular, tongue. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes. A biological sample is typically obtained from a eukaryotic organism, such as insects, protozoa, birds, fish, reptiles, and preferably a mammal such as rat, mouse, cow, dog, guinea pig, or rabbit, and most preferably a primate such as chimpanzees or humans. Preferred tissues include tongue tissue, isolated taste buds, and testis tissue.

The phrase "functional effect" in the context of assays for testing compounds that modulate TC-ICS-mediated taste transduction includes the determination of any parameter that is indirectly or directly under the influence of TC-ICS. It includes changes in ion flux, membrane potential, current flow, transcription, G-protein binding, GPCR phosphorylation or dephosphorylation, signal transduction, receptor-ligand interactions, second messenger concentrations (*e.g.*, cAMP, IP_3, or intracellular Ca^{2+}), *in vitro, in vivo,* and *ex vivo* and also includes other physiologic effects such increases or decreases of neurotransmitter or hormone release.

A "pharmacologically effective amount of a composition that modulates taste signaling by an ion channel subunit" is an amount of a composition (which may

consist of a single chemical compound or a mixture of chemical compounds, preferably combined with a carrier such as a solvent) that is effective to detectably alter a measurable property or a functional effect of a TC-ICS. The TC-ICS may be in solution, or expressed in a naturally occurring cell, in a tissue cultured cell, in a recombinant cell, or in a wild type or recombinant organism. In a main embodiment, the TC-ICS is present at least at the surface membrane of a cell which may be in a tissue culture or in a tissue of a live multicellular organism, especially a mammal. The precise value of the effective amount varies according to the compound and the species, age, sex, condition and health of the cells or organism that contains the TC-ICS. In solution, typically the effective amount is an amount sufficient to yield a concentration of at least 10 nM – 10 mM, preferably at least 0.1 μM to 1 mM, and more preferably 10 to 100 μM. When administered to a subject, the effective amount is from about 1 ng/kg to 10 mg/kg for a typical subject.

"Determining the functional effect" denotes assays for a compound that increases or decreases a parameter that is indirectly or directly under the influence of TC-ICS. Such functional effects are measured by any means known to those skilled in the art, *e.g.*, patch clamping, voltage-sensitive dyes, whole cell currents, radioisotope efflux, inducible markers, oocyte or tissue culture cell expression of TC-ICS; transcriptional activation of TC-ICS; ligand binding assays; voltage, membrane potential and conductance changes; ion flux assays; changes in intracellular second messengers such as cAMP and inositol triphosphate (IP_3); changes in intracellular calcium levels; neurotransmitter release, and the like.

"Inhibitors," "activators," and "modulators" of TC-ICS refer to inhibitory or activating molecules identified using *in vitro* and *in vivo* assays for taste transduction, *e.g.*, ligands, agonists, antagonists, and their homologs and mimetics. Inhibitors are compounds that decrease, block, prevent, delay activation, inactivate, desensitize, or down regulate taste transduction, *e.g.*, antagonists. Activators are compounds that increase, open, activate, facilitate, enhance activation, sensitize or up regulate taste transduction, *e.g.*, agonists. Modulators include genetically modified versions of TC-ICS, *e.g.*, with altered activity, as well as naturally occurring and synthetic ligands, antagonists, agonists, small chemical molecules and the like. Such assays for inhibitors and activators include, *e.g.*, expressing TC-ICS in cells or cell membranes, applying

putative modulator compounds, and then determining the functional effects on taste transduction, as described above. Compounds identified by these assays are typically combined with food or medicine and used to alter its taste to the subject (mammalian, preferably a human) who ingests it.

Samples or assays comprising TC-ICS that are treated with a potential activator, inhibitor, or modulator are compared to control samples without the inhibitor, activator, or modulator to examine the extent of inhibition. Control samples (untreated with inhibitors) are assigned a relative TC-ICS activity value of 100%. Inhibition of TC-ICS is achieved when the TC-ICS activity value relative to the control is about 80%, preferably 50%, more preferably 25-1%. Activation of TC-ICS is achieved when the TC-ICS activity value relative to the control (untreated with activators) is 110%, more preferably 150%, more preferably 200-500%, more preferably 1000-3000% higher.

The terms "isolated," "purified" or "biologically pure" refer to material that is substantially or essentially free from components which normally accompany it as found in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified. In particular, an isolated TC-ICS nucleic acid is separated from open reading frames that flank the TC-ICS gene and encode proteins other than TC-ICS. The term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the nucleic acid or protein is at least 85% pure, more preferably at least 95% pure, and most preferably at least 99% pure.

"Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon

substitutions) and complementary sequences, as well as the sequence explicitly indicated. The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an analog or mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. Polypeptides are modified, *e.g.*, by the addition of carbohydrate residues to form glycoproteins. The terms "polypeptide," "peptide" and "protein" include glycoproteins, as well as non-glycoproteins.

The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group., *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes (A, T, G, C, U, etc.).

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more

selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260:2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes* 8:91-98 (1994)). Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon in an amino acid herein, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants and alleles of the invention.

The following groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);
2) Serine (S), Threonine (T);
3) Aspartic acid (D), Glutamic acid (E);
4) Asparagine (N), Glutamine (Q);
5) Cysteine (C), Methionine (M);
6) Arginine (R), Lysine (K), Histidine (H);
7) Isoleucine (I), Leucine (L), Valine (V); and
8) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

(*see, e.g.*, Creighton, Proteins (1984) for a discussion of amino acid properties).

A "label" or a "detectable moiety" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P, ^{45}Ca, fluorescent groups, molecules or dyes, electron-dense reagents, enzymes (*e.g.*, as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins for which specific detectable ligands (such as antibodies) exist or can be made (*e.g.*, by incorporating a radiolabel into the ligand).

A "labeled nucleic acid probe or oligonucleotide" is one that is bound, either covalently, through a linker or a chemical bond, or noncovalently, through ionic, van der Waals, electrostatic, or hydrogen bonds to a label such that the presence of the probe may be detected by detecting the presence of the label bound to the probe.

As used herein a "nucleic acid probe or oligonucleotide" is defined as a nucleic acid capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation. As used herein, a probe may include natural (*i.e.*, A, G, C, or T) or modified bases (7-deazaguanosine, inosine, etc.). In addition, the bases in a probe may be joined by a linkage other than a phosphodiester bond, so long as it does not interfere with hybridization. Thus, for example, probes may be peptide nucleic acids in which the constituent bases are joined by peptide bonds rather than phosphodiester linkages. It will be understood by one of skill in the art that probes may bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. The probes are preferably directly labeled as with isotopes, chromophores, lumiphores, chromogens, or indirectly labeled such as with biotin to which a streptavidin complex may later bind. By assaying for the presence or absence of the probe, one can detect the presence or absence of the select sequence or subsequence.

The term "recombinant" when used with reference, *e.g.*, to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within

the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, *e.g.*, a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (*e.g.*, a fusion protein).

A "promoter" is defined as an array of nucleic acid control sequences that direct transcription of a nucleic acid. As used herein, a promoter includes necessary nucleic acid sequences near the start site of transcription, such as in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

An "expression vector" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a host cell. The expression vector can be part of a plasmid, virus, or nucleic acid fragment. Typically, the expression vector includes a nucleic acid to be transcribed operably linked to a promoter.

The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said

to be "substantially identical." This definition also refers to the complement of a test sequence. Preferably, the percent identity exists over a region of the sequence that is at least about 25 amino acids in length, more preferably over a region that is 50 or 100 amino acids in length.

For sequence comparison, one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A "comparison window," as used herein, includes reference to a segment of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection.

One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.* 35:351-360 (1987). The method used is similar to the method described by Higgins & Sharp, *CABIOS* 5:151-153 (1989). The program can align up to 300 sequences, each of a maximum length of 5,000 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences,

producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. Using PILEUP, a reference sequence is compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps. PILEUP can be obtained from the GCG sequence analysis software package, *e.g.*, version 7.0 (Devereaux *et al.*, *Nuc. Acids Res.* 12:387-395 (1984)).

Another example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci.* USA 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA*

90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below.

The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (*e.g.*, total cellular or library DNA or RNA).

The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10° C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium

ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60° C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42° C, or, 5x SSC, 1% SDS, incubating at 65° C, with wash in 0.2x SSC, and 0.1% SDS at 65° C.

Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37° C, and a wash in 1X SSC at 45° C. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

A further indication that two polynucleotides are substantially identical is if the reference sequence, amplified by a pair of oligonucleotide primers, can then be used as a probe under stringent hybridization conditions to isolate the test sequence from a cDNA or genomic library, or to identify the test sequence in, *e.g.*, a northern or Southern blot. Alternatively, another indication that the sequences are substantially identical is if the same set of PCR primers can be used to amplify both sequences.

"Antibody" refers to a polypeptide encoded by an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each

pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

Antibodies exist, *e.g.*, as intact immunoglobulins or as a number of well characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)'$_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The F(ab)'$_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)'$_2$ dimer into an Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region (*see Fundamental Immunology* (Paul ed., 3d ed. 1993)). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies or those synthesized *de novo* using recombinant DNA methodologies (*e.g.*, single chain Fv).

A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, *e.g.*, an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

For preparation of monoclonal or polyclonal antibodies, any technique known in the art can be used (*see, e.g.*, Kohler & Milstein, *Nature* 256:495-497 (1975); Kozbor *et al.*, *Immunology Today* 4:72 (1983); Cole *et al.*, pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985)). Techniques for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies. Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab

fragments that specifically bind to selected antigens (*see, e.g.*, McCafferty *et al., Nature* 348, 552-554 (1990); Marks *et al., Biotechnology* 10, 779-783 (1992)).

An "TC-ICS" antibody is an antibody or antibody fragment that specifically binds a polypeptide encoded by the TC-ICS gene, cDNA, or a subsequence thereof.

The term "immunoassay" is an assay that uses an antibody to specifically bind an antigen. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the antigen.

The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised to TC-ICS from specific species such as rat, mouse, or human can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with TC-ICS and not with other proteins, except for polymorphic variants and alleles of TC-ICS. This selection may be achieved by subtracting out antibodies that cross-react with TC-ICS molecules from other species. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g.*, Harlow & Lane, *Antibodies, A Laboratory Manual* (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

The phrase "selectively associates with" refers to the ability of a nucleic acid to "selectively hybridize" with another as defined above, or the ability of an antibody to "selectively (or specifically) bind" to a protein, as defined above.

III. Assays for taste modulation

A. Assays for taste cell-specific ion channel subunit activity

TC-ICS and its alleles, interspecies homologs, and polymorphic variants participate in taste transduction. The activity of TC-ICS polypeptides can be assessed using a variety of *in vitro* and *in vivo* assays, *e.g.*, measuring second messenger (*e.g.*, cAMP, cGMP, IP_3, DAG, or Ca^{2+}), ion flux, phosphorylation levels, transcription levels, neurotransmitter levels, and the like. Furthermore, such assays can be used to screen for activators, inhibitors, and modulators of TC-ICS. Such activators, inhibitors, and modulators of taste transduction activity are useful for customizing taste.

Biologically active TC-ICS polypeptides, either recombinant or naturally occurring, are used to screen activators, inhibitors, or modulators of taste. The TC-ICS polypeptides are isolated, *e.g.*, expressed in a cell, expressed in a membrane derived from a cell, expressed in tissue or in an animal, either recombinant or naturally occurring. For example, tongue slices, dissociated cells from a tongue, transformed cells, or membranes are used. Taste transduction can also be examined *in vitro* with soluble or solid state reactions. Preferably, TC-ICS of the assay will be selected from a polypeptide having a sequence of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8, or conservatively modified variant thereof. Alternatively, TC-ICS of the assay will be derived from a eukaryote and includes an amino acid subsequence having amino acid sequence identity to SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8. Generally, the amino acid sequence identity will be at least 70%, preferably at least 85%, most preferably at least 90-95%.

Samples or assays that are treated with a test compound which potentially activates, inhibits, or modulates TC-ICS are compared to control samples that are not treated without the test compound, to examine the extent of modulation. Control samples (untreated with activators, inhibitors, or modulators) are assigned a relative TC-ICS activity value of 100%. Inhibition of TC-ICS is achieved when the TC-ICS activity value relative to the control is about 90% (*e.g.*, 10% less than the control), preferably 50%, more preferably 25-5%, most preferably 5-0%. Activation of TC-ICS is achieved when the TC-ICS activity value relative to the control is 110% (*e.g.*, 10% more than the control), more preferably 150%, more preferably 200-500%, more preferably 1000-2000%, or more than 2000% (*e.g.*, 10,000%).

In one embodiment, the activity of TC-ICS polypeptides is assessed by measuring, *e.g.*, changes in intracellular second messengers, such as cAMP, cGMP, IP_3,

DAG, or Ca^{2+}. Therefore, the second messenger levels are used as reporters for potential activators, inhibitors, and modulators of TC-ICS polypeptides.

Ion channel modulation typically initiates or inhibits subsequent intracellular events via, *e.g.*, G-proteins and/or other enzymes, such as adenylate cyclase or phospholipase C, which are downstream from the ion channel-mediated events in taste transduction pathways. For example, ion channel activation may result in a change in the level of intracellular cyclic nucleotides, *e.g.*, cAMP or cGMP, by activating or inhibiting enzymes such as adenylate cyclase by G-protein α and $\beta\gamma$ subunits. These intracellular cyclic nucleotides, in turn, may modulate other molecules, such as, cyclic nucleotide-gated ion channels, *e.g.*, channels that are made permeable to cations by binding of cAMP or cGMP (*see, e.g.*, Altenhofen *et al., Proc. Natl. Acad. Sci. U.S.A.* 88:9868-9872 (1991) and Dhallan *et al., Nature* 347:184-187 (1990)). Cells for this type of assay are made by co-transfection of a host cell with any one or a combination of DNA encoding a cyclic nucleotide-gated ion channel, GPCR phosphatase, DNA encoding TC-ICS, and DNA encoding a G-protein coupled receptor. The receptor may be, *e.g.*, metabotropic glutamate receptors, muscarinic acetylcholine receptors, dopamine receptors, serotonin receptors, and the like, which, when activated, causes a change in cyclic nucleotide levels in the cytoplasm.

In response to external stimuli, certain ion channels may activate other effectors, such as phospholipase C, through G-proteins, GPCRs, modulating enzyme activities, or other ion channels. Activation of phospholipase C results in the production of inositol 1, 4, 5-triphosphate (IP_3) and diacylglycerol (DAG) from inositol 4,5-biphosphate (PIP_2) (Berridge & Irvine, *Nature* 312:315-21 (1984)). IP_3 in turn stimulates the release of intracellular calcium ion stores. Cells may exhibit increased cytoplasmic calcium levels as a result of contribution from both intracellular stores and via activation of ion channels, in which case it may be desirable although not necessary to conduct such assays in calcium-free buffer, optionally supplemented with a chelating agent such as EGTA, to distinguish fluorescence response resulting from calcium release from internal stores. Thus, a change in the level of second messengers, such as IP_3, DAG, or Ca^{2+} can be used to assess TC-ICS function. Furthermore, a change in the level of these second messengers are used to screen for activators, inhibitors, and modulators of TC-ICS polypeptides.

In one embodiment, the changes in intracellular cAMP or cGMP are measured using immunoassays. The method described in Offermanns & Simon, *J. Biol. Chem.* 270:15175-15180 (1995) may be used to determine the level of cAMP. Also, the method described in Felley-Bosco *et al., Am. J. Resp. Cell and Mol. Biol.* 11:159-164 (1994) may be used to determine the level of cGMP. Further, an assay kit for measuring cAMP and/or cGMP is described in U.S. Patent 4,115,538, herein incorporated by reference.

In another embodiment, phosphatidyl inositol (PI) hydrolysis are analyzed according to U.S. Patent 5,436,128, herein incorporated by reference. Briefly, the assay involves labeling of cells with ^{3}H-myoinositol for 48 or more hrs. The labeled cells are treated with a test compound for one hour. The treated cells are lysed and extracted in chloroform-methanol-water after which the inositol phosphates were separated by ion exchange chromatography and quantified by scintillation counting. Fold stimulation is determined by calculating the ratio of cpm in the presence of agonist to cpm in the presence of buffer control. Likewise, fold inhibition is determined by calculating the ratio of cpm in the presence of antagonist to cpm in the presence of buffer control (which may or may not contain an agonist).

In another embodiment, intracellular Ca^{2+} levels are analyzed, *e.g.*, using fluorescent Ca^{2+} indicator dyes and fluorometric imaging (*see, e.g.*, Hall *et al., Nature* 331:729 (1988); Kudo *et al., Neuros.* 50:619-625 (1992); van Heugten *et al., J. Mol. Cell. Cardiol.* 26:1081-93 (1994)).

In another embodiment, the activity of TC-ICS can also be assessed by measuring changes in ion flux. Changes in ion flux may be measured by determining changes in polarization (*i.e.*, electrical potential) of the cell or membrane expressing TC-ICS. One means to determine changes in cellular polarization is by measuring changes in current (thereby measuring changes in polarization) with voltage-clamp and patch-clamp techniques, *e.g.*, the "cell-attached" mode, the "inside-out" mode, and the "whole cell" mode (*see, e.g.*, Ackerman *et al., New Engl. J. Med.* 336:1575-1595 (1997)). Whole cell currents are conveniently determined using the standard methodology (*see, e.g.*, Hamil *et al., PFlugers. Archiv.* 391:85 (1981)). Other known assays include: radiolabeled ion flux assays and fluorescence assays using voltage-sensitive dyes (*see, e.g.*, Vestergarrd-Bogind *et al., J. Membrane Biol.* 88:67-75 (1988); Gonzales & Tsien, *Chem. Biol.* 4:269-277 (1997); Daniel *et al., J. Pharmacol. Meth.* 25:185-193 (1991); Holevinsky *et al., J.

Membrane Biology 137:59-70 (1994)). A method for the whole-cell recording from non-dissociated taste cells within mouse taste bud is described in Miyamoto *et al.*, *J. Neurosci Methods* 64:245-252 (1996). Therefore, changes in ion flux are used to screen for activators, inhibitors, and modulators of TC-ICS. Generally, the compounds to be tested are present in the range from 1 pM to 100 mM.

Assays for measuring changes in ion flux include cells that are loaded with ion or voltage sensitive dyes to report TC-ICS activity. Assays for determining activity of these polypeptides can also use known agonists and antagonists for these polypeptides as negative or positive controls to assess activity of tested compounds. In assays for identifying modulatory compounds (*e.g.*, agonists, antagonists), changes in the level of ions in the cytoplasm or membrane voltage will be monitored using an ion sensitive or membrane voltage fluorescent indicator, respectively. Among the ion-sensitive indicators and voltage probes that may be employed are those disclosed in the Molecular Probes 1997 Catalog.

In another embodiment, phosphorylation of taste cell specific proteins are measured to assess the effects of a test compound on TC-ICS function. This can be achieved by using a method disclosed in, *e.g.*, U.S. Patent 5,834,216, herein incorporated by reference. A duplicate cell culture containing expressed TC-ICS is prepared. One of the duplicate cultures is exposed to a test compound. Cell lysates from the duplicate cultures are prepared. The cell lysates are contacted with ATP wherein the ATP has a gamma-phosphate having a detectable label, or an analog of a gamma phosphate (*i.e.*, having a label capable of being transferred to a phosphorylation site such as gamma S^{35}). The level of phosphorylated taste cell specific proteins may be measured by precipitating the cell lysates with an antibody specific for taste cell specific proteins. After precipitation, phosphorylated (labeled) taste cell specific proteins may be separated from other cellular proteins by electrophoresis or by chromatographic methods. By way of example, labeled taste cell specific proteins may be separated on denaturing polyacrylamide gels after which the separated proteins may be transferred to, for example, a nylon or nitrocellulose membrane followed by exposure to X-ray film. Relative levels of phosphorylation are then determined after developing the exposed X-ray film and quantifying the density of bands corresponding to the taste cell specific proteins, for example, densitometry. The autoradiograph may also be used to localize the bands on the membrane corresponding to labeled taste cell specific proteins after which

they may be excised from the membrane and counted by liquid scintillation or other counting methods. Using this method, a test compound which effects the function of TC-ICS is identified by its ability to increase or decrease phosphorylation of taste cell specific proteins compared to control cells not exposed to the test compound.

In another embodiment, transcription levels are measured to assess the effects of a test compound on TC-ICS function. A host cell containing TC-ICS is contacted with a test compound for a sufficient time to effect any interactions, and then the level of TC-ICS gene expression is measured. The amount of time to effect such interactions may be empirically determined, such as by running a time course and measuring the level of transcription as a function of time. The amount of transcription may be measured by using any method known to those of skill in the art to be suitable. For example, mRNA expression of TC-ICS may be detected using northern blots or their polypeptide products may be identified using immunoassays. Alternatively, transcription based assays using reporter gene may be used as described in U.S. Patent 5,436,128, herein incorporated by reference. The reporter genes can be, *e.g.*, chloramphenicol acetyltransferase, firefly luciferase, bacterial luciferase, β-galactosidase and alkaline phosphatase. Furthermore, TC-ICS can be used as indirect reporters via attachment to a second reporter such as green fluorescent protein (*see*, *e.g.*, Mistili & Spector, *Nature Biotechnology* 15:961-964 (1997)).

The amount of transcription is then compared to the amount of transcription in either the same cell in the absence of the test compound, or it may be compared with the amount of transcription in a substantially identical cell that lacks TC-ICS. A substantially identical cell may be derived from the same cells from which the recombinant cell was prepared but which had not been modified by introduction of heterologous DNA. Any difference in the amount of transcription indicates that the test compound has in some manner altered the activity of TC-ICS.

Other physiological change that affects TC-ICS activity are used to assess the influence of a test compound on the polypeptides of this invention. When the functional consequences are determined using intact cells or animals, one can also measure a variety of effects such as transmitter release, hormone release, transcriptional changes to both known and uncharacterized genetic markers (*e.g.*, northern blots), changes in cell metabolism such as cell growth or pH changes, and the like.

In one preferred embodiment, TC-ICS activity is measured by expressing TC-ICS in a heterologous cell with a taste cell specific G-protein receptor (TC-GPCR; *see* U.S.S.N. 60/094,465 filed July 28, 1998; U.S.S.N. 60/095,464 filed July 28, 1998; U.S.S.N. 60/112,747 filed December 17, 1998) and a promiscuous G-protein that links the receptor to a phospholipase C signal transduction pathway (*see* Offermanns & Simon, *J. Biol. Chem.* 270:15175-15180 (1995); *see also* Example II). A TC-GPCR, such as GPCR-B3 or GPCR-B4, can be used in the assays (*see* U.S.S.N. 60/094,465 filed July 28, 1998 for the description of GPCR-B3 and U.S.S.N. 60/095,464 filed July 28, 1998 and 60/112,747 filed December 17, 1998 for the description of GPCR-B4). Gα14 or Gα15 can be used as a promiscuous G-protein alpha subunit (Wilkie *et al., PNAS USA* 88:10049-10053 (1991)). Such promiscuous G-proteins allow coupling of a wide range of receptors. Alternatively, a taste cell specific G-protein alpha subunit can be used, such as the Gα subunit described in copending application U.S.S.N. 60/117,367, TTC ref. no. 02307E-092600, filed 1/27/99, and U.S.S.N. 60/117,404, TTC ref. no. 02307E-092700, filed 1/27/99, herein incorporated by reference. Preferably the cell line is HEK-293 (which does not naturally express GPCR-B4) and the promiscuous G-protein is Gα15 (Offermanns & Simon, *supra*). Modulation of taste transduction is assayed by measuring changes in intracellular Ca^{2+} levels. Changes in Ca^{2+} levels are preferably measured using fluorescent Ca^{2+} indicator dyes and fluorometric imaging.

B. Modulators

The compounds tested as modulators of TC-ICS can be an ion, any small chemical compound, or a biological entity, such as a protein (*e.g.*, a GPCR or a GPCR binding protein), sugar, nucleic acid or lipid. Alternatively, modulators can be genetically altered versions of TC-ICS. Typically, test compounds will be small chemical molecules and peptides. Essentially any chemical compound can be used as a potential modulator or ligand in the assays of the invention, although most often compounds that can be dissolved in aqueous or organic (especially DMSO-based) solutions are used. The assays are designed to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (*e.g.*, in microtiter formats on microtiter plates in robotic assays). It will be appreciated that there are many suppliers of chemical compounds, including Sigma (St.

Louis, MO), Aldrich (St. Louis, MO), Sigma-Aldrich (St. Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs Switzerland) and the like.

In one preferred embodiment, high throughput screening methods involve providing a combinatorial chemical or peptide library containing a large number of potential therapeutic compounds (potential modulator or ligand compounds). Such "combinatorial chemical libraries" or "ligand libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as conventional "lead compounds" or can themselves be used as potential or actual therapeutics.

A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (amino acids) in every possible way for a given compound length (*i.e.*, the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (*see, e.g.*, U.S. Patent 5,010,175, Furka, *Int. J. Pept. Prot. Res.* 37:487-493 (1991) and Houghton *et al.*, *Nature* 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptoids (*e.g.*, PCT Publication No. WO 91/19735), encoded peptides (*e.g.*, PCT Publication No. WO 93/20242), random bio-oligomers (*e.g.*, PCT Publication No. WO 92/00091), benzodiazepines (*e.g.*, U.S. Pat. No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs *et al.*, *Proc. Nat. Acad. Sci. USA* 90:6909-6913 (1993)), vinylogous polypeptides (Hagihara *et al.*, *J. Amer. Chem. Soc.* 114:6568 (1992)), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann *et al.*, *J. Amer. Chem. Soc.* 114:9217-9218 (1992)), analogous organic syntheses of small compound libraries (Chen *et al.*, *J. Amer. Chem. Soc.* 116:2661 (1994)), oligocarbamates (Cho *et al.*, *Science* 261:1303 (1993)), and/or peptidyl phosphonates (Campbell *et al.*, *J. Org. Chem.* 59:658 (1994)), nucleic acid libraries (*see*

Ausubel, Berger and Sambrook, all *supra*), peptide nucleic acid libraries (*see, e.g.,* U.S. Patent 5,539,083), antibody libraries (*see, e.g.,* Vaughn *et al., Nature Biotechnology,* 14(3):309-314 (1996) and PCT/US96/10287), carbohydrate libraries (*see, e.g.,* Liang *et al., Science,* 274:1520-1522 (1996) and U.S. Patent 5,593,853), small organic molecule libraries (*see, e.g.,* benzodiazepines, Baum C&EN, Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337; benzodiazepines, 5,288,514, and the like).

Devices for the preparation of combinatorial libraries are commercially available (*see, e.g.,* 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA). In addition, numerous combinatorial libraries are themselves commercially available (*see, e.g.,* ComGenex, Princeton, N.J., Asinex, Moscow, Ru, Tripos, Inc., St. Louis, MO, ChemStar, Ltd, Moscow, RU, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, etc.).

In one embodiment, the invention provides solid phase based *in vitro* assays in a high throughput format, where the cell or tissue expressing TC-ICS is attached to a solid phase substrate. In the high throughput assays of the invention, it is possible to screen up to several thousand different modulators or ligands in a single day. In particular, each well of a microtiter plate is used to run a separate assay against a selected potential modulator, or, if concentration or incubation time effects is to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay about 100 (*e.g.*, 96) modulators. If 1536 well plates are used, then a single plate can easily assay from about 100- about 1500 different compounds. It is possible to assay several different plates per day; assay screens for up to about 6,000-20,000 different compounds is possible using the integrated systems of the invention. More recently, microfluidic approaches to reagent manipulation have been developed, *e.g.*, by Caliper Technologies (Palo Alto, CA).

C. Computer-based assays

Yet another assay for compounds that modulate TC-ICS activity involves computer assisted drug design, in which a computer system is used to generate a three-dimensional structure of TC-ICS based on the structural information encoded by the

amino acid sequence. The input amino acid sequence interacts directly and actively with a pre-established algorithm in a computer program to yield secondary, tertiary, and quaternary structural models of the protein. The models of the protein structure are then examined to identify regions of the structure that have the ability to bind, *e.g.*, ligands. These regions are then used to identify ligands that bind to the protein.

The three-dimensional structural model of the protein is generated by entering amino acid sequences of at least 10 amino acid residues that are present in IC-ICS (for example, SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8 or conservatively modified versions thereof) or corresponding nucleic acid sequences encoding a TC-ICS polypeptide (for example, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:7 or conservatively modified versions thereof) into a computer system.. The amino acid sequence represents the primary sequence or subsequence of the protein, which encodes the structural information of the protein. At least 10 residues of the amino acid sequence (or a nucleotide sequence encoding 10 amino acids) are entered into the computer system from computer keyboards, computer readable substrates that include, but are not limited to, electronic storage media (*e.g.*, magnetic diskettes, tapes, cartridges, and chips), optical media (*e.g.*, CD ROM), information distributed by internet sites, and by RAM. The three-dimensional structural model of the protein is then generated by the interaction of the amino acid sequence and the computer system, using software known to those of skill in the art. The three-dimensional structural model of the protein is saved to a computer readable form and be used for further analysis (*e.g.*, identifying potential ligand binding regions of the protein and screening for mutations, alleles and interspecies homologs of the gene).

The amino acid sequence represents a primary structure that encodes the information necessary to form the secondary, tertiary and quaternary structure of the protein of interest. The software looks at certain parameters encoded by the primary sequence to generate the structural model. These parameters are referred to as "energy terms," and primarily include electrostatic potentials, hydrophobic potentials, solvent accessible surfaces, and hydrogen bonding. Secondary energy terms include van der Waals potentials. Biological molecules form the structures that minimize the energy terms in a cumulative fashion. The computer program is therefore using these terms encoded by the primary structure or amino acid sequence to create the secondary structural model.

The tertiary structure of the protein encoded by the secondary structure is then formed on the basis of the energy terms of the secondary structure. The user at this point can enter additional variables such as whether the protein is membrane bound or soluble, its location in the body, and its cellular location, *e.g.*, cytoplasmic, surface, or nuclear. These variables along with the energy terms of the secondary structure are used to form the model of the tertiary structure. In modeling the tertiary structure, the computer program matches hydrophobic faces of secondary structure with like, and hydrophilic faces of secondary structure with like.

Once the structure has been generated, potential ligand binding regions are identified by the computer system. Three-dimensional structures for potential ligands are generated by entering amino acid or nucleotide sequences or chemical formulas of compounds, as described above. The three-dimensional structure of the potential ligand is then compared to that of the TC-ICS protein to identify ligands that bind to TC-ICS. Binding affinity between the protein and ligands is determined using energy terms to determine which ligands have an enhanced probability of binding to the protein. The results, such as three-dimensional structures for potential ligands and binding affinity of ligands, can also be saved to a computer readable form and is used for further analysis (*e.g.*, generating a three dimensional model of mutated proteins having an altered binding affinity for a ligand).

Computer systems are also used to screen for mutations, polymorphic variants, alleles and interspecies homologs of TC-ICS genes. Such mutations are associated with disease states or genetic traits. As described above, high density oligonucleotide arrays (GeneChip™) and related technology can also be used to screen for mutations, polymorphic variants, alleles and interspecies homologs. Once the variants are identified, diagnostic assays are used to identify patients having such mutated genes. Identification of the mutated TC-ICS genes involves receiving input of a first nucleic acid or amino acid sequence encoding selected from the group consisting of, *e.g.*, SEQ ID NOS: 1, 4 or 7, or SEQ ID NOS: 2, 5 or 8, and conservatively modified versions thereof. The sequence is entered into the computer system as described above and then saved to a computer readable form. The first nucleic acid or amino acid sequence is then compared to a second nucleic acid or amino acid sequence that has substantial identity to the first sequence. The second sequence is entered into the computer system in the manner

described above. Once the first and second sequences are compared, nucleotide or amino acid differences between the sequences are identified. Such sequences can represent allelic differences in TC-ICS genes, and mutations associated with disease states and genetic traits.

III. Isolation of the nucleic acid encoding TC-ICS

A. General recombinant DNA methods

This invention relies on routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this invention include Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., (1994)).

For nucleic acids, sizes are given in either kilobases (kb) or base pairs (bp). These are estimates derived from agarose or acrylamide gel electrophoresis, from sequenced nucleic acids, or from published DNA sequences. For proteins, sizes are given in kilodaltons (kDa) or amino acid residue numbers. Proteins sizes are estimated from gel electrophoresis, from sequenced proteins, from derived amino acid sequences, or from published protein sequences.

Oligonucleotides that are not commercially available can be chemically synthesized according to the solid phase phosphoramidite triester method first described by Beaucage & Caruthers, *Tetrahedron Letts.* 22:1859-1862 (1981), using an automated synthesizer, as described in Van Devanter *et. al.*, *Nucleic Acids Res.* 12:6159-6168 (1984). Purification of oligonucleotides is by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson & Reanier, *J. Chrom.* 255:137-149 (1983).

The sequence of the cloned genes and synthetic oligonucleotides can be verified after cloning using, *e.g.*, the chain termination method for sequencing double-stranded templates of Wallace *et al.*, *Gene* 16:21-26 (1981).

B. Cloning methods for the isolation of nucleotide sequences encoding TC-ICS

In general, the nucleic acid sequences encoding TC-ICS and related nucleic acid sequence homologs are cloned from cDNA and genomic DNA libraries by

hybridization with a probe, or isolated using amplification techniques with oligonucleotide primers. For example, TC-ICS sequences are typically isolated from mammalian nucleic acid (genomic or cDNA) libraries by hybridizing with a nucleic acid probe, the sequence of which can be derived from SEQ ID NOS: 1, 3, 4, 6, or 7. A suitable tissue from which TC-ICS and cDNA can be isolated is tongue tissue, preferably taste bud tissue, more preferably individual taste cells. For example, circumvallate, foliate, fungiform taste receptor cells are used to isolate RNA and cDNA.

Amplification techniques using primers are also used to amplify and isolate TC-ICS from DNA or RNA (*see, e.g.*, Dieffenfach & Dveksler, *PCR Primer: A Laboratory Manual* (1995)). These primers are used, *e.g.*, to amplify either the full length sequence or a probe of one to several hundred nucleotides, which is then used to screen a mammalian library for full-length TC-ICS.

Nucleic acids encoding TC-ICS can also be isolated from expression libraries using antibodies as probes. Such polyclonal or monoclonal antibodies can be raised using the sequence of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8.

TC-ICS polymorphic variants, alleles, and interspecies homologs that are substantially identical to TC-ICS are isolated using TC-ICS nucleic acid probes and oligonucleotides under stringent hybridization conditions, by screening libraries. Alternatively, expression libraries are used to clone TC-ICS and its polymorphic variants, alleles, and interspecies homologs, by detecting expressed homologs immunologically with antisera or purified antibodies made against TC-ICS which also recognize and selectively bind to the TC-ICS homolog.

To make a cDNA library, one should choose a source that is rich in TC-ICS mRNA, *e.g.*, tongue tissue, or isolated taste buds. The mRNA is then made into cDNA using reverse transcriptase, ligated into a recombinant vector, and transfected into a recombinant host for propagation, screening and cloning. Methods for making and screening cDNA libraries are well known (*see, e.g.*, Gubler & Hoffman, *Gene* 25:263-269 (1983); Sambrook *et al., supra*; Ausubel *et al., supra*).

For a genomic library, the DNA is extracted from the tissue and either mechanically sheared or enzymatically digested to yield fragments of about 12-20 kb. The fragments are then separated by gradient centrifugation from undesired sizes and are constructed in bacteriophage lambda vectors. These vectors and phage are packaged *in vitro*. Recombinant phage are analyzed by plaque hybridization as described in Benton &

Davis, *Science* 196:180-182 (1977). Colony hybridization is carried out as generally described in Grunstein *et al.*, *Proc. Natl. Acad. Sci. USA.*, 72:3961-3965 (1975).

An alternative method of isolating TC-ICS nucleic acid and its homologs combines the use of synthetic oligonucleotide primers and amplification of an RNA or DNA template (*see* U.S. Patents 4,683,195 and 4,683,202; *PCR Protocols: A Guide to Methods and Applications* (Innis *et al.*, eds, 1990)). Methods such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) are used to amplify nucleic acid sequences of TC-ICS directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. Degenerate oligonucleotides can be designed to amplify TC-ICS homologs using the sequences provided herein. Restriction endonuclease sites can be incorporated into the primers. Polymerase chain reaction or other *in vitro* amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of TC-ICS encoding mRNA in physiological samples, for nucleic acid sequencing, or for other purposes. Genes amplified by the PCR reaction can be purified from agarose gels and cloned into an appropriate vector.

Gene expression of TC-ICS can also be analyzed by techniques known in the art, *e.g.*, reverse transcription and amplification of mRNA, isolation of total RNA or poly A^+ RNA, northern blotting, dot blotting, *in situ* hybridization, RNase protection, and the like. In one embodiment, high density oligonucleotide arrays technology (*e.g.*, GeneChip™) is used to identify homologs and polymorphic variants of the TC-ICS of the invention (*see, e.g.*, Gunthand *et al.*, *AIDS Res. Hum. Retroviruses* 14:869-876 (1998); Kozal *et al.*, *Nat. Med.* 2:753-759 (1996); Matson *et al.*, *Anal. Biochem.* 224:101-106 (1995); Lockhart *et al.*, *Nat. Biotechnol.* 14:1675-1680 (1996); Gingeras *et al.*, *Genome Res.* 8:435-448 (1998); Hacia *et al.*, *Nucleic Acids Res.* 26:3865-3866 (1998)).

Synthetic oligonucleotides are used to construct recombinant TC-ICS genes for use as probes or for expression of protein. This method is performed using a series of overlapping oligonucleotides usually 40-120 bp in length, representing both the sense and nonsense strands of the gene. These DNA fragments are then annealed, ligated and cloned. Alternatively, amplification techniques are used with precise primers to amplify a specific subsequence of the TC-ICS nucleic acid. The specific subsequence is then ligated into an expression vector.

The nucleic acid encoding TC-ICS is typically cloned into intermediate vectors before transformation into prokaryotic or eukaryotic cells for replication and/or expression. These intermediate vectors are typically prokaryote vectors, *e.g.*, plasmids, or shuttle vectors.

C. Expression in prokaryotes and eukaryotes

To obtain high level expression of a cloned gene or nucleic acid, such as those cDNAs encoding TC-ICS, one typically subclones TC-ICS into an expression vector that contains a strong promoter to direct transcription, a transcription/translation terminator, and if for a nucleic acid encoding a protein, a ribosome binding site for translational initiation. Suitable bacterial promoters are well known in the art and described, *e.g.*, in Sambrook *et al.* and Ausubel *et al.* Bacterial expression systems for expressing the TC-ICS proteins are available in, *e.g.*, *E. coli, Bacillus sp.,* and *Salmonella* (Palva *et al.*, *Gene* 22:229-235 (1983); Mosbach *et al.*, *Nature* 302:543-545 (1983)). Kits for such expression systems are commercially available. Eukaryotic expression systems for mammalian cells, yeast, and insect cells are well known in the art and are also commercially available.

The promoter used to direct expression of a heterologous nucleic acid depends on the particular application. The promoter is preferably positioned about the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

In addition to the promoter, the expression vector typically contains a transcription unit or expression cassette that contains all the additional elements required for the expression of the TC-ICS encoding nucleic acid in host cells. A typical expression cassette thus contains a promoter operably linked to the nucleic acid sequence encoding TC-ICS and signals required for efficient polyadenylation of the transcript, ribosome binding sites, and translation termination. The nucleic acid sequence encoding TC-ICS may typically be linked to a cleavable signal peptide sequence to promote secretion of the encoded protein by the transformed cell. Such signal peptides would include, among others, the signal peptides from tissue plasminogen activator, insulin, and neuron growth factor, and juvenile hormone esterase of *Heliothis virescens*. Additional elements of the

cassette may include enhancers and, if genomic DNA is used as the structural gene, introns with functional splice donor and acceptor sites.

In addition to a promoter sequence, the expression cassette should also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes.

The particular expression vector used to transport the genetic information into the cell is not particularly critical. Any of the conventional vectors used for expression in eukaryotic or prokaryotic cells may be used. Standard bacterial expression vectors include plasmids such as pBR322 based plasmids, pSKF, pET23D, and fusion expression systems such as GST and LacZ. Epitope tags can also be added to recombinant proteins to provide convenient methods of isolation, *e.g.*, c-myc.

Expression vectors containing regulatory elements from eukaryotic viruses are typically used in eukaryotic expression vectors, *e.g.*, SV40 vectors, papilloma virus vectors, and vectors derived from Epstein-Barr virus. Other exemplary eukaryotic vectors include pMSG, pAV009/A$^+$, pMTO10/A$^+$, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV40 early promoter, SV40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

Some expression systems have markers that provide gene amplification such as thymidine kinase, hygromycin B phosphotransferase, and dihydrofolate reductase. Alternatively, high yield expression systems not involving gene amplification are also suitable, such as using a baculovirus vector in insect cells, with a TC-ICS encoding sequence under the direction of the polyhedrin promoter or other strong baculovirus promoters.

The elements that are typically included in expression vectors also include a replicon that functions in *E. coli*, a gene encoding antibiotic resistance to permit selection of bacteria that harbor recombinant plasmids, and unique restriction sites in nonessential regions of the plasmid to allow insertion of eukaryotic sequences. The particular antibiotic resistance gene chosen is not critical, any of the many resistance genes known in the art are suitable. The prokaryotic sequences are preferably chosen

such that they do not interfere with the replication of the DNA in eukaryotic cells, if necessary.

Standard transfection methods are used to produce bacterial, mammalian, yeast or insect cell lines that express large quantities of TC-ICS, which are then purified using standard techniques (*see, e.g.*, Colley *et al.*, *J. Biol. Chem.* 264:17619-17622 (1989); *Guide to Protein Purification*, in *Methods in Enzymology*, vol. 182 (Deutscher, ed., 1990)). Transformation of eukaryotic and prokaryotic cells are performed according to standard techniques (*see, e.g.*, Morrison, *J. Bact.* 132:349-351 (1977); Clark-Curtiss & Curtiss, *Methods in Enzymology* 101:347-362 (Wu *et al.*, eds, 1983)).

Any of the well known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, polybrene, protoplast fusion, electroporation, liposomes, microinjection, plasma vectors, viral vectors and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (*see, e.g.*, Sambrook *et al.*, *supra*). It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one gene into the host cell capable of expressing TC-ICS.

After the expression vector is introduced into the cells, the transfected cells are cultured under conditions favoring expression of TC-ICS, which is recovered from the culture using standard techniques identified below.

IV. Purification of TC-ICS

Either naturally occurring or recombinant TC-ICS can be purified for use in functional assays. Preferably, recombinant TC-ICS is purified. Naturally occurring TC-ICS is purified, *e.g.*, from mammalian tissue such as tongue tissue, and any other source of a TC-ICS homolog. Recombinant TC-ICS is purified from any suitable expression system.

TC-ICS may be purified to substantial purity by standard techniques, including selective precipitation with such substances as ammonium sulfate; column chromatography, immunopurification methods, and others (*see, e.g.*, Scopes, *Protein Purification: Principles and Practice* (1982); U.S. Patent No. 4,673,641; Ausubel *et al.*, *supra*; and Sambrook *et al.*, *supra*).

A number of procedures can be employed when recombinant TC-ICS is being purified. For example, proteins having established molecular adhesion properties can be reversible fused to TC-ICS. With the appropriate ligand, TC-ICS can be selectively adsorbed to a purification column and then freed from the column in a relatively pure form. The fused protein is then removed by enzymatic activity. Finally TC-ICS could be purified using immunoaffinity columns.

A. Purification of TC-ICS *from recombinant bacteria*

Recombinant proteins are expressed by transformed bacteria in large amounts, typically after promoter induction; but expression can be constitutive. Promoter induction with IPTG is a one example of an inducible promoter system. Bacteria are grown according to standard procedures in the art. Fresh or frozen bacteria cells are used for isolation of protein.

Proteins expressed in bacteria may form insoluble aggregates ("inclusion bodies"). Several protocols are suitable for purification of TC-ICS inclusion bodies. For example, purification of inclusion bodies typically involves the extraction, separation and/or purification of inclusion bodies by disruption of bacterial cells, *e.g.*, by incubation in a buffer of 50 mM TRIS/HCL pH 7.5, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 0.1 mM ATP, and 1 mM PMSF. The cell suspension can be lysed using 2-3 passages through a French Press, homogenized using a Polytron (Brinkman Instruments) or sonicated on ice. Alternate methods of lysing bacteria are apparent to those of skill in the art (*see, e.g.*, Sambrook *et al., supra*; Ausubel *et al., supra*).

If necessary, the inclusion bodies are solubilized, and the lysed cell suspension is typically centrifuged to remove unwanted insoluble matter. Proteins that formed the inclusion bodies may be renatured by dilution or dialysis with a compatible buffer. Suitable solvents include, but are not limited to urea (from about 4 M to about 8 M), formamide (at least about 80%, volume/volume basis), and guanidine hydrochloride (from about 4 M to about 8 M). Some solvents which are capable of solubilizing aggregate-forminG-proteins, for example SDS (sodium dodecyl sulfate), 70% formic acid, are inappropriate for use in this procedure due to the possibility of irreversible denaturation of the proteins, accompanied by a lack of immunogenicity and/or activity. Although guanidine hydrochloride and similar agents are denaturants, this denaturation is not irreversible and renaturation may occur upon removal (by dialysis, for example) or

dilution of the denaturant, allowing re-formation of immunologically and/or biologically active protein. Other suitable buffers are known to those skilled in the art. TC-ICS is separated from other bacterial proteins by standard separation techniques, *e.g.*, with Ni-NTA agarose resin.

Alternatively, it is possible to purify TC-ICS from bacteria periplasm. After lysis of the bacteria, when TC-ICS is exported into the periplasm of the bacteria, the periplasmic fraction of the bacteria can be isolated by cold osmotic shock in addition to other methods known to skill in the art. To isolate recombinant proteins from the periplasm, the bacterial cells are centrifuged to form a pellet. The pellet is resuspended in a buffer containing 20% sucrose. To lyse the cells, the bacteria are centrifuged and the pellet is resuspended in ice-cold 5 mM $MgSO_4$ and kept in an ice bath for approximately 10 minutes. The cell suspension is centrifuged and the supernatant decanted and saved. The recombinant proteins present in the supernatant can be separated from the host proteins by standard separation techniques well known to those of skill in the art.

B. Standard protein separation techniques for purifying TC-ICS

<u>Solubility fractionation</u>

Often as an initial step, particularly if the protein mixture is complex, an initial salt fractionation can separate many of the unwanted host cell proteins (or proteins derived from the cell culture media) from the recombinant protein of interest. The preferred salt is ammonium sulfate. Ammonium sulfate precipitates proteins by effectively reducing the amount of water in the protein mixture. Proteins then precipitate on the basis of their solubility. The more hydrophobic a protein is, the more likely it is to precipitate at lower ammonium sulfate concentrations. A typical protocol includes adding saturated ammonium sulfate to a protein solution so that the resultant ammonium sulfate concentration is between 20-30%. This concentration will precipitate the most hydrophobic of proteins. The precipitate is then discarded (unless the protein of interest is hydrophobic) and ammonium sulfate is added to the supernatant to a concentration known to precipitate the protein of interest. The precipitate is then solubilized in buffer and the excess salt removed if necessary, either through dialysis or diafiltration. Other methods that rely on solubility of proteins, such as cold ethanol precipitation, are well known to those of skill in the art and can be used to fractionate complex protein mixtures.

Size differential filtration

The molecular weight of TC-ICS are used to isolate them from proteins of greater and lesser size using ultrafiltration through membranes of different pore size (for example, Amicon or Millipore membranes). As a first step, the protein mixture is ultrafiltered through a membrane with a pore size that has a lower molecular weight cut-off than the molecular weight of the protein of interest. The retentate of the ultrafiltration is then ultrafiltered against a membrane with a molecular cut off greater than the molecular weight of the protein of interest. The recombinant protein will pass through the membrane into the filtrate. The filtrate can then be chromatographed as described below.

Column chromatography

TC-ICS can also be separated from other proteins on the basis of its size, net surface charge, hydrophobicity, and affinity for ligands. In addition, antibodies raised against proteins can be conjugated to column matrices and the proteins immunopurified. All of these methods are well known in the art. It will be apparent to one of skill that chromatographic techniques are performed at any scale and using equipment from many different manufacturers (*e.g.*, Pharmacia Biotech).

V. Immunological detection of TC-ICS

In addition to the detection of TC-ICS genes and gene expression using nucleic acid hybridization technology, one can also use immunoassays to detect TC-ICS, *e.g.*, to identify taste receptor cells and variants of TC-ICS. Immunoassays can be used to qualitatively or quantitatively analyze TC-ICS. A general overview of the applicable technology can be found in Harlow & Lane, *Antibodies: A Laboratory Manual* (1988).

A. Antibodies to TC-ICS

Methods of producing polyclonal and monoclonal antibodies that react specifically with TC-ICS are known to those of skill in the art (*see, e.g.*, Coligan, *Current Protocols in Immunology* (1991); Harlow & Lane, *supra*; Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed. 1986); and Kohler & Milstein, *Nature* 256:495-497 (1975). Such techniques include antibody preparation by selection of antibodies from libraries of recombinant antibodies in phage or similar vectors, as well as

preparation of polyclonal and monoclonal antibodies by immunizing rabbits or mice (*see, e.g.*, Huse *et al.*, *Science* 246:1275-1281 (1989); Ward *et al.*, *Nature* 341:544-546 (1989)).

A number of TC-ICS-comprising immunogens may be used to produce antibodies specifically reactive with TC-ICS. For example, recombinant TC-ICS or an antigenic fragment thereof, is isolated as described herein. Recombinant protein are expressed in eukaryotic or prokaryotic cells as described above, and purified as generally described above. Recombinant protein is the preferred immunogen for the production of monoclonal or polyclonal antibodies. Alternatively, a synthetic peptide derived from the sequences disclosed herein and conjugated to a carrier protein can be used as an immunogen. Naturally occurring TS-ICS may also be used either in pure or impure form. The product is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies are generated, for subsequent use in immunoassays to measure the protein.

Methods of production of polyclonal antibodies are known to those of skill in the art. An inbred strain of mice (*e.g.*, BALB/C mice) or rabbits is immunized with the protein using a standard adjuvant, such as Freund's adjuvant, and a standard immunization protocol. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to TC-ICS. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein can be done if desired (*see* Harlow & Lane, *supra*).

Monoclonal antibodies may be obtained by various techniques familiar to those skilled in the art. Briefly, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (*see* Kohler & Milstein, *Eur. J. Immunol.* 6:511-519 (1976)). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a

binding fragment thereof by screening a DNA library from human B cells according to the general protocol outlined by Huse *et al.*, *Science* 246:1275-1281 (1989).

Monoclonal antibodies and polyclonal sera are collected and titered against the immunogen protein in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Typically, polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against non-TC-ICS proteins or even other related proteins from other organisms, using a competitive binding immunoassay. Specific polyclonal antisera and monoclonal antibodies will usually bind with a K_d of at least about 0.1 mM, more usually at least about 1 μM, preferably at least about 0.1 μM or better, and most preferably, 0.01 μM or better.

Once TC-ICS specific antibodies are available, TC-ICS are detected by a variety of immunoassay methods. For a review of immunological and immunoassay procedures, *see Basic and Clinical Immunology* (Stites & Terr eds., 7th ed. 1991). Moreover, the immunoassays of the present invention are performed in any of several configurations, which are reviewed extensively in *Enzyme Immunoassay* (Maggio, ed., 1980); and Harlow & Lane, *supra*.

B. Immunological binding assays

TC-ICS are detected and/or quantified using any of a number of well recognized immunological binding assays (*see, e.g.*, U.S. Patents 4,366,241; 4,376,110; 4,517,288; and 4,837,168). For a review of the general immunoassays, *see also*, *Methods in Cell Biology: Antibodies in Cell Biology*, volume 37 (Asai, ed. 1993); *Basic and Clinical Immunology* (Stites & Terr, eds., 7th ed. 1991). Immunological binding assays (or immunoassays) typically use an antibody that specifically binds to a protein or antigen of choice (in this case the TC-ICS or antigenic subsequence thereof). The antibody (*e.g.*, anti-TC-ICS) may be produced by any of a number of means well known to those of skill in the art and as described above.

Immunoassays also often use a labeling agent to specifically bind to and label the complex formed by the antibody and antigen. The labeling agent may itself be one of the moieties comprising the antibody/antigen complex. Thus, the labeling agent may be a labeled polypeptide of TC-ICS or a labeled anti-TC-ICS antibody. Alternatively, the labeling agent may be a third moiety, such a secondary antibody, that

specifically binds to the antibody/TC-ICS complex (a secondary antibody is typically specific to antibodies of the species from which the first antibody is derived). Other proteins capable of specifically binding immunoglobulin constant regions, such as protein A or protein G may also be used as the label agent. These proteins exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species (*see, e.g.*, Kronval *et al.*, *J. Immunol.* 111:1401-1406 (1973); Akerstrom *et al.*, *J. Immunol.* 135:2539-2542 (1985)). The labeling agent can be modified with a detectable moiety, such as biotin, to which another molecule can specifically bind, such as streptavidin. A variety of detectable moieties are well known to those skilled in the art.

Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, preferably from about 5 minutes to about 24 hours. However, the incubation time will depend upon the assay format, antigen, volume of solution, concentrations, and the like. Usually, the assays will be carried out at ambient temperature, although they can be conducted over a range of temperatures, such as 10°C to 40°C.

Non-competitive assay formats

Immunoassays for detecting TC-ICS in samples may be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of antigen is directly measured. In one preferred "sandwich" assay, for example, the anti-TC-ICS antibodies can be bound directly to a solid substrate on which they are immobilized. These immobilized antibodies then capture TC-ICS present in the test sample. TC-ICS is thus immobilized is then bound by a labeling agent, such as a second TC-ICS antibody bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second or third antibody is typically modified with a detectable moiety, such as biotin, to which another molecule specifically binds, *e.g.*, streptavidin, to provide a detectable moiety.

Competitive assay formats

In competitive assays, the amount of TC-ICS present in the sample is measured indirectly by measuring the amount of a known, added (exogenous) TC-ICS displaced (competed away) from an anti-TC-ICS antibody by the unknown TC-ICS present in a sample. In one competitive assay, a known amount of TC-ICS is added to a sample and the sample is then contacted with an antibody that specifically binds to TC-ICS. The amount of exogenous TC-ICS bound to the antibody is inversely proportional to the concentration of TC-ICS present in the sample. In a particularly preferred embodiment, the antibody is immobilized on a solid substrate. The amount of TC-ICS bound to the antibody may be determined either by measuring the amount of TC-ICS present in a TC-ICS/antibody complex, or alternatively by measuring the amount of remaining uncomplexed protein. The amount of TC-ICS may be detected by providing a labeled TC-ICS molecule.

A hapten inhibition assay is another preferred competitive assay. In this assay the known TC-ICS is immobilized on a solid substrate. A known amount of anti-TC-ICS antibody is added to the sample, and the sample is then contacted with the immobilized TC-ICS. The amount of anti-TC-ICS antibody bound to the known immobilized TC-ICS is inversely proportional to the amount of TC-ICS present in the sample. Again, the amount of immobilized antibody may be detected by detecting either the immobilized fraction of antibody or the fraction of the antibody that remains in solution. Detection may be direct where the antibody is labeled or indirect by the subsequent addition of a labeled moiety that specifically binds to the antibody as described above.

Cross-reactivity determinations

Immunoassays in the competitive binding format can also be used for cross-reactivity determinations. For example, a protein at least partially encoded by SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8 can be immobilized to a solid support. Proteins (*e.g.*, TC-ICS proteins and homologs) are added to the assay that compete for binding of the antisera to the immobilized antigen. The ability of the added proteins to compete for binding of the antisera to the immobilized protein is compared to the ability of TC-ICS encoded by SEQ ID NOS: 1, 3, 4, 6 or 7 to compete with itself. The percent cross-reactivity for the above proteins is calculated, using standard calculations. Those

antisera with less than 10% cross-reactivity with each of the added proteins listed above are selected and pooled. The cross-reacting antibodies are optionally removed from the pooled antisera by immunoabsorption with the added considered proteins, *e.g.*, distantly related homologs.

The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described above to compare a second protein, thought to be perhaps an allele or polymorphic variant of TC-ICS to the immunogen protein (*i.e.*, TC-ICS of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8). In order to make this comparison, the two proteins are each assayed at a wide range of concentrations and the amount of each protein required to inhibit 50% of the binding of the antisera to the immobilized protein is determined. If the amount of the second protein required to inhibit 50% of binding is less than 10 times the amount of the protein encoded by SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8 that is required to inhibit 50% of binding, then the second protein is said to specifically bind to the polyclonal antibodies generated to a TC-ICS immunogen.

Other assay formats

Western blot (immunoblot) analysis is used to detect and quantify the presence of TC-ICS in the sample. The technique generally comprises separating sample proteins by gel electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support, (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the sample with the antibodies that specifically bind TC-ICS. The anti-TC-ICS antibodies specifically bind to the TC-ICS on the solid support. These antibodies may be directly labeled or alternatively may be subsequently detected using labeled antibodies (*e.g.*, labeled sheep anti-mouse antibodies) that specifically bind to the anti-TC-ICS antibodies.

Other assay formats include liposome immunoassays (LIA), which use liposomes designed to bind specific molecules (*e.g.*, antibodies) and release encapsulated reagents or markers. The released chemicals are then detected according to standard techniques (*see* Monroe *et al.*, *Amer. Clin. Prod. Rev.* 5:34-41 (1986)).

Reduction of non-specific binding

One of skill in the art will appreciate that it is often desirable to minimize non-specific binding in immunoassays. Particularly, where the assay involves an antigen or antibody immobilized on a solid substrate it is desirable to minimize the amount of non-specific binding to the substrate. Means of reducing such non-specific binding are well known to those of skill in the art. Typically, this technique involves coating the substrate with a proteinaceous composition. In particular, protein compositions such as bovine serum albumin (BSA), nonfat powdered milk, and gelatin are widely used with powdered milk being most preferred.

Labels

The particular label or detectable group used in the assay is not a critical aspect of the invention, as long as it does not significantly interfere with the specific binding of the antibody used in the assay. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and, in general, most any label useful in such methods can be applied to the present invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include magnetic beads (*e.g.*, DYNABEADS™), fluorescent dyes (*e.g.*, fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (*e.g.*, ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic beads (*e.g.*, polystyrene, polypropylene, latex, etc.).

The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on sensitivity required, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (*e.g.*, biotin) is covalently bound to the molecule. The ligand then binds to another molecules (*e.g.*, streptavidin) molecule, which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent

compound, or a chemiluminescent compound. The ligands and their targets can be used in any suitable combination with antibodies that recognize TC-ICS, or secondary antibodies that recognize anti-TC-ICS.

The molecules can also be conjugated directly to signal generating compounds, *e.g.*, by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidotases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, etc. Chemiluminescent compounds include luciferin, and 2,3-dihydrophthalazinediones, *e.g.*, luminol. For a review of various labeling or signal producing systems that may be used, see U.S. Patent No. 4,391,904.

Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence may be detected visually, by means of photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Finally simple colorimetric labels may be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of the target antibody is detected by simple visual inspection.

VI. Kits

TC-ICS and its homologs are a useful tool for identifying taste receptor cells, for forensics and paternity determinations, and for examining taste transduction (*e.g.*, generating a topographical map between the taste cells of the tongue and the

corresponding taste centers in the brain). Specific reagents that specifically hybridize to TC-ICS nucleic acid, such as its probes and primers, and specific reagents that specifically bind to the TC-ICS protein, *e.g.*, their antibodies are used to examine taste cell expression and taste transduction regulation.

Nucleic acid assays for the presence of TC-ICS DNA and RNA in a sample include numerous techniques are known to those skilled in the art, such as Southern analysis, northern analysis, dot blots, RNase protection, high density oligonucleotide arrays, S1 analysis, amplification techniques such as PCR and LCR, and *in situ* hybridization. In *in situ* hybridization, for example, the target nucleic acid is liberated from its cellular surroundings in such as to be available for hybridization within the cell while preserving the cellular morphology for subsequent interpretation and analysis (see Example I). The following articles provide an overview of the art of *in situ* hybridization: Singer *et al., Biotechniques* 4:230-250 (1986); Haase *et al., Methods in Virology*, vol. VII, pp. 189-226 (1984); and *Nucleic Acid Hybridization: A Practical Approach* (Hames *et al.*, eds. 1987). In addition, TC-ICS protein can be detected with the various immunoassay techniques described above. The test sample is typically compared to both a positive control (*e.g.*, a sample expressing recombinant TC-ICS) and a negative control.

The present invention also provides for kits for screening for modulators of TC-ICS. Such kits can be prepared from readily available materials and reagents. For example, such kits can comprise any one or more of the following materials: TC-ICS, reaction tubes, and instructions for testing TC-ICS activity. Preferably, the kit contains biologically active TC-ICS. A wide variety of kits and components can be prepared according to the present invention, depending upon the intended user of the kit and the particular needs of the user.

VII. Administration and pharmaceutical compositions

Taste modulators can be administered directly to the mammalian subject for modulation of taste *in vivo*. Administration is by any of the routes normally used for introducing a modulator compound into ultimate contact with the tissue to be treated, preferably the tongue or mouth. The taste modulators are administered in any suitable manner, preferably with pharmaceutically acceptable carriers. Suitable methods of administering such modulators are available and well known to those of skill in the art,

and, although more than one route can be used to administer a particular composition, a particular route can often provide a more immediate and more effective reaction than another route.

Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions of the present invention (*see, e.g., Remington's Pharmaceutical Sciences*, 17th ed. 1985)).

The taste modulators, alone or in combination with other suitable components, can be made into aerosol formulations (*i.e.*, they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Formulations suitable for administration include aqueous and non-aqueous solutions, isotonic sterile solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, by orally, topically, intravenously, intraperitoneally, intravesically or intrathecally. Preferably, the compositions are administered orally or nasally. The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials. Solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. The modulators can also be administered as part a of prepared food or drug.

The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial response in the subject over time. The dose will be determined by the efficacy of the particular taste modulators employed and the condition of the subject, as well as the body weight or surface area of the area to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular compound or vector in a particular subject.

In determining the effective amount of the modulator to be administered in a physician may evaluate circulating plasma levels of the modulator, modulator toxicities,

and the production of anti-modulator antibodies. In general, the dose equivalent of a modulator is from about 1 ng/kg to 10 mg/kg for a typical subject.

For administration, taste modulators of the present invention can be administered at a rate determined by the LD-50 of the modulator, and the side-effects of the inhibitor at various concentrations, as applied to the mass and overall health of the subject. Administration can be accomplished via single or divided doses.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

EXAMPLES

The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

Example I: Taste cell specific expression of TC-ICS and cloning

Taste bud isolation:

Subtraction libraries made from rat circumvallate cells were used to isolate the TC-ICS nucleic acids of the invention. Briefly, single taste receptor cells were isolated from dissociated circumvallate papillae from the rat tongue as generally described by Bernhardt *et al.*, *J. Physiol.* (Lond), 490, 325-336 (1996). Amplified single cell cDNA was Southern and dot-blotted and probed with radiolabeled probes to identify potentially similar cell types. Gustducin, a G-protein specifically expressed in a subset of taste receptor cells was chosen as a marker for taste cells (McLaughlin *et al.*, *Nature*

357:563-569 (1992)). Tubulin and N-Cam were chosen to confirm the integrity of the cells and validate the amplification reactions. Bacteriophage lambda cDNA libraries were then constructed from individual Gustducin positive cells and were plated at low density on LB/Agar plates.

Subtraction and library generation:

Total RNA was extracted from circumvallate papillae using standard procedures(Trizol method; GibcoBRL); approximately 2 μg of Poly A+ RNA were purified using Qiagen's Oligotex mRNA Kit and used to prime cDNA synthesis following standard protocols. Samples were stored in media containing RNAse inhibitors to prevent degradation of mRNA.

Suppression subtraction was performed according to Clontech's PCR-Select cDNA Subtraction protocol using circumvallate cDNA as tester and non-taste cDNA prepared from non-taste lingual tissue as driver. The efficiency of the subtraction procedure was monitored by probing the subtracted product with two known taste-specific genes (Lunch and Repeater); both of these genes were enriched greater than 50 – fold in the subtracted cDNA compared to the unsubtracted circumvallate cDNA.

Sequence analysis and in situ hybridization:

Subtracted cDNAs were cloned into pBluescript-based plasmid vectors to generate a subtracted cDNA library. 930 clones from the subtracted library were then chosen for sequence analysis. DNA sequences were mined using Blast searches against nucleotide and protein databases (http://www.ncbi.nlm.nih.gov/BLAST/). All sequences were also analyzed for the presence of potential transmembrane segments (http://dot.imgen.bcm.tmc.edu:9331/seq-search/struc-predict.html). cDNAs encoding novel sequences were used in *in situ* hybridizations to tongue tissue sections to examine taste cell expression.

Results

Clone 501-PCR46 showed selective expression in subsets of taste receptor cells of circumvallate and fungiform papillae. This clone was chosen for detailed characterization, including full-length cDNA isolation and sequencing. The results show

that Clone 501-PCR46 represents a mRNA specifically expressed in circumvallate, foliate and fungiform taste receptor cells . This is a relatively rare cDNA found in approximately 1/100,000 cDNAs from an oligo-dT primed circumvallate cDNA library (Hoon *et al.*), *Cell* 96: 541-51(1999) and in approximately 1 /300 cDNAs in our subtracted library. The cDNA sequence from clone 501-PCR46 is shown in SEQ ID NO:1 and the predicted amino acid is in SEQ ID NO:2. The corresponding *in situ* hybridization to a tissue section demonstrating taste cell specificity is shown in Figure 1.

Clone 501-PCR46 encodes a novel member of the TRP family of ion channels (Harteneck *et al.*, "*From worm to man: three subfamilies of TRP channels*," *Trends Neurosci.* 23(4):159-66(2000)). A related gene known as Mtr1 was previously reported to be associated with chromosomal imprinting (Enklaar *et al.* "*Mtr1, a novel biallelically expressed gene in the center of the mouse distal chromosome 7 imprinting cluster, is a member of the Trp gene family,*" *Genomics.* 67(2):179-87; Esswein *et al.*, 2000)), and a chromosomal interval implicated in the Beckwith-Wiedemann syndrome region on human 11p15.5 (Yatsuki *et al.* "*Sequence-based structural features between Kvlqt1 and Tapa1 on mouse chromosome 7F4/F5 corresponding to the Beckwith-Wiedemann syndrome region on human 11p15.5:. long-stretches of unusually well conserved intronic sequences of kvlqt1 between mouse and human.*" *DNA Res.* 7(3):195-206 (2000); Paulsen *et al.*, "*Sequence conservation and variability of imprinting in the Beckwith-Wiedemann syndrome gene cluster in human and mouse,*" *Hum Mol Genet.* 9(12):1829-41 (2000)). However, no function, cell-type specific expression, or role had been assigned to this "orphan" ion channel. Clone 501-PCR46 appears to be a splice variant of Mtr1 (aka LTRPC5; Paulsen *et al.*, 2000), and is a component of the taste signaling machinery.

Example II: Functional Analyses of TC-ICS proteins expressed in a heterologous cell

TC-ICS encoding nucleic acids (e.g., SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:7) are expressed in a heterologous cell, alone or with other cell transduction proteins such as a G-protein α subunit and/ or a taste cell specific G-protein coupled receptor such as GPCR-B3, GPCR-B4 (*see* U.S.S.N. 60/094,465 filed July 28, 1998 for the description of GPCR-B3 and U.S.S.N. 60/095,464 filed July 28, 1998 and 60/112,747 filed December 17, 1998 for the description of GPCR-B4), Gα14 or Gα15 (Wilkie *et al.*,

PNAS USA 88:10049-10053 (1991)). These transformed cells are used to screen for activators, inhibitors, and modulators of TC-ICS, including mdulatorts of its intewraction with GPCRs or G-proteins. Different assays for ion channel mediated functions are performed as generally described above and in PCT 99/06307, incorporated by reference herein.

In particular, modulation of taste transduction is assayed by measuring changes in intracellular Ca^{2+} levels, which change in response to modulation of the TC-ICS signal transduction pathway via administration of a molecule that associates with TC-ICS. Changes in Ca^{2+} levels are preferably measured using fluorescent Ca^{2+} indicator dyes and fluorometric imaging. The amount of $[Ca^{2+}]_i$ is then compared to the amount of $[Ca^{2+}]_i$ in either the same cell in the absence of the test compound, or it may be compared to the amount of $[Ca^{2+}]_i$ in a substantially identical cell that lacks TC-ICS.

WHAT IS CLAIMED IS:

1. A method for identifying a compound that modulates taste signaling in taste cells, the method comprising the steps of:

(i) contacting the compound with a eukaryotic host cell or cell membrane which expresses a taste cell-specific ion channel subunit:

(a) having greater than about 70% amino acid sequence identity to a polypeptide having a sequence selected from the group that consists of SEQ ID NO: 2, SEQ ID NO: 5, and SEQ ID NO: 8; and

(b) specifically binding to polyclonal antibodies that specifically bind to a polypeptide having a sequence selected from the group that consists of SEQ ID NO: 2, SEQ ID NO: 5, and SEQ ID NO: 8; and

(ii) determining a functional effect of the compound upon a transmembrane ion flux of a predetermined ion, thereby identifying a compound that modulates taste signaling in taste cells.

2. The method of claim1, wherein the functional effect is determined by measuring changes in intracellular ion concentration.

3. The method of claim 1, wherein the functional effect is determined by measuring changes in intracellular Ca^{++}.

4. The method of claim 1, wherein the changes in ion flux are measured by an assay selected from the group consisting of a voltage clamp assay, a patch clamp assay, a radiolabeled ion flux assay, or a fluorescence assay using ion sensitive dyes.

5. The method of claim 1, wherein the cell or cell membrane is attached to a solid substrate.

6. The method of claim 1, wherein the taste cell-specific ion channel subunit is from a mammal.

7. The method of claim 6, wherein the taste cell-specific ion channel subunit has an amino acid sequence selected from the group that consists of SEQ ID NO: 2, SEQ ID NO: 5, and SEQ ID NO: 8.

8. The method of claim 1, wherein the host cell is a human cell.

9. The method of claim 1, wherein the host cell is a HEK 293 cell.

10. A method for identifying a compound that binds to a taste cell-specific ion channel subunit, said method comprising:

(i) contacting control cells that do not express a taste cell-specific ion channel subunit with a test compound;

(ii) contacting test cells with said test compound, wherein said test cells are transformed with and express a nucleic acid encoding a taste cell-specific ion channel subunit

(a) having greater than about 70% amino acid sequence identity to to a polypeptide having a sequence selected from the group that consists of SEQ ID NO: 2, SEQ ID NO: 5, and SEQ ID NO: 8; and

(b) specifically binding to polyclonal antibodies that specifically bind to a polypeptide having a sequence selected from the group that consists of SEQ ID NO: 2, SEQ ID NO: 5, and SEQ ID NO: 8; and;

(iii) identifying test compounds that bind to said taste cell-specific ion channel subunit by comparing the amount of said test compound that binds to said test cells to the amount of said test compound that binds to said control cells.

11. A method according to claim 10 wherein said nucleic acid encodes an amino acid selected from the group that consists of SEQ ID NO: 2, SEQ ID NO: 5, and SEQ ID NO: 8.

12. A method of modulating taste signaling in taste cells of an individual, comprising

administering to an individual a pharmacologically effective amount of a composition that modulates taste signalling by an ion channel subunit

(a) having greater than about 70% amino acid sequence identity to to a polypeptide having a sequence selected from the group that consists of SEQ ID NO: 2, SEQ ID NO: 5, and SEQ ID NO: 8; and

(b) specifically binding to polyclonal antibodies that specifically bind to a polypeptide having a sequence selected from the group that consists of SEQ ID NO: 2, SEQ ID NO: 5, and SEQ ID NO: 8;

thereby modulating taste signalling in taste cells of said individual.

13. A method of claim 12, wherein said ion channel subunit has an amino acid sequence selected from the group that consists of SEQ ID NO: 2, SEQ ID NO: 5, and SEQ ID NO: 8.

14. A method of claim 12, wherein said individual is a mammal.

15. A method of claim 12, wherein said individual is a human.

TGCCGTGGCTTCTACCAGGATGGGCGCAGGATGGAGAAGAGAGGGCCACC
CAAGCGGCCTGCAGGCCAGAAATGGCTGCCGGACCTCAGTCGGAAGAGTG
AAGACCCATGGAGGGACCTGTTCCTTTGGGCTGTGCTGCAGAACCGTTAT
GAGATGGCCACATACTTCTGGGCCATGGGCCGGGAGGGTGTGGCTGCTGC
TCTGGCGGCCTGCAAGATCATCAAGGAAATGTCCCACCTGGAGAAAGAGG
CAGAGGTGGCCCGCACTATGCGTGAGGCCAAGTATGAGCAGCTGGCCCTC
GATCTTTTCTCAGAGTGCTACAGCAACAGTGAGGACCGTGCCTTTGCCCT
GTTGGTGCGCAGGAACCACAGCTGGAGCAGGACCACCTGCCTGCACCTGG
CCACTGAGGCCGATGCCAAGGCCTTCTTTGCCCATGATGGTGTGCAAGCA
TTCCTGACGAAGATCTGGTGGGGAGACATGGCCACAGGCACACCCATCTT
ACGACTTCTGGGTGCCTTCACCTGCCCAGCCCTCATCTACACAAATCTCA
TCTCCTTCAGTGAGGATGCCCCGCAGAGGATGGACCTGGAAGATCTGCAG
GAGCCAGACAGTTTGGATATGGAAAAGAGCTTCCTGTGCAGCCATGGTGG
CCAATTGGAGAAGTTAACAGAGGCGCCAAGGGCTCCTGGCGATCTAGGCC
CACAAGCTGCCTTCCTGCTCACACGGTGGAGGAAGTTCTGGGGCGCTCCT
GTGACTGTGTTCTTGGGGAATGTGGTCATGTACTTTGCATTCCTCTTCCT
ATTCTCCTACGTCCTGCTGGTGGATTTCAGGCCACCACCCCAGGGGCCAT
CTGGGTCGGAAGTTACCCTGTATTTCTGGGTCTTCACACTGGTGCTGGAG
GAAATCCGACAGGGATTCTTCACAAACGAGGACACCCGTCTGGTGAAGAA
GTTCACTCTGTACGTAGAAGACAACTGGAACAAATGTGACATGGTGGCCA
TCTTCCTGTTCATTGTTGGTGTCACCTGTAGAATGGTGCCCTCCGTGTTT
GAGGCTGGCCGGACTGTTCTGGCCATTGACTTCATGGTGTTCACACTTCG
GCTCATCCACATCTTTGCTATTCACAAGCAGCTGGGTCCTAAGATCATCA
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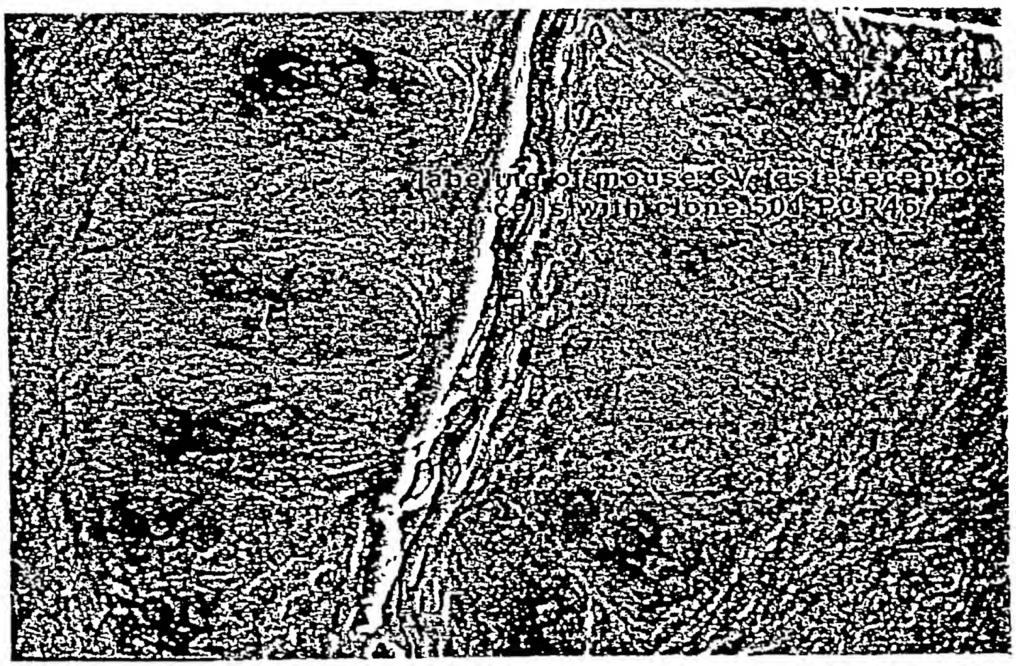

SUBSTITUTE SHEET (RULE 26)

SEQUENCE LISTING

SEQ ID NO:1

Rat L-TRP taste cDNA sequence

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SEQ ID NO:2

Rat L-TRP taste predicted protein sequence

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SEQ ID NO:3

Mouse genomic sequence of the region that contains ltrpc5 (gi|8574073|emb|AJ251835.1|MMU251835 Mus musculus Kcnq1, Ltrpc5, Mash2, Tapa-1, Tssc4 and Tssc6 genes, alternative transcripts)

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SEQ ID NO:4

Mouse ltrpc5 cDNA sequence

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SEQ ID NO:5

Predicted mouse ltrpc5 amino acid sequence

(translation from GI9754868)

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SEQ ID NO:6

Human genomic squence of the region that contains ltrpc5

gi|3687269|gb|AC003693.1|AC003693 Human Chromosome 11p15.5 PAC clone pDJ915f1 containing KvLQT1 gene, complete sequence [Homo sapiens]

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SEQ ID NO:7

Human ltrpc5 (mtr1) cDNA sequence

gi|6715116|gb|AF177473.1|AF177473 Homo sapiens MTR1 (MTR1) mRNA, complete cds, alternatively spliced

GAGGCCACCATGCAGGATGTCCAAGGCCCCCGTCCCGGAAGCCCCGGGGATG
CTGAAGACCGGCGGGAGCTGGGCTTGCACAGGGGCGAGGTCAACTTTGGAGG
GTCTGGGAAGAAGCGAGGCAAGTTTGTACGGGTGCCGAGCGGAGTGGCCCCG
TCTGTGCTCTTTGACCTGCTGCTTGCTGAGTGGCACCTGCCGGCCCCCAACCT
GGTGGTGTCCCTGGTGGGTGAGGAGCAGCCTTTCGCCATGAAGTCCTGGCTGC
GGGATGTGCTGCGCAAGGGGCTGGTGAAGGCGGCTCAGAGCACAGGAGCCT
GGATCCTGACCAGTGCCCTCCGCGTGGGCCTGGCCAGGCATGTCGGGCAGGC
CGTGCGCGACCACTCGCTGGCCAGCACGTCCACCAAGGTCCGTGTGGTTGCTG
TCGGCATGGCCTCGCTGGGCCGCGTCCTGCACCGCCGCATTCTGGAGGAGGC
CCAGGAGGATTTTCCTGTCCACTACCCTGAGGATGACGGCGGCAGCCAGGGC
CCCCTCTGTTCACTGGACAGCAACCTCTCCCACTTCATCCTGGTGGAGCCAGG
CCCCCCGGGGAAGGGCGATGGGCTGACGGAGCTGCGGCTGAGGCTGGAGAA
GCACATCTCGGAGCAGAGGGCGGGCTACGGGGGCACTGGCAGCATCGAGATC
CCTGTCCTCTGCTTGCTGGTCAATGGTGATCCCAACACCTTGGAGAGGATCTC
CAGGGCCGTGGAGCAGGCTGCCCCGTGGCTGATCCTGGTAGGCTCGGGGGGC
ATCGCCGATGTGCTTGCTGCCCTAGTGAACCAGCCCCACCTCCTGGTGCCCAA
GGTGGCCGAGAAGCAGTTTAAGGAGAAGTTCCCCAGCAAGCATTTCTCTTGG
GAGGACATCGTGCGCTGGACCAAGCTGCTGCAGAACATCACCTCACACCAGC
ACCTGCTCACCGTGTATGACTTCGAGCAGGAGGGCTCCGAGGAGCTGGACAC
GGTCATCCTGAAGGCGCTGGTGAAAGCCTGCAAGAGCCACAGCCAGGAGCCT
CAGGACTATCTGGATGAGCTCAAGCTGGCCGTGGCCTGGGACCGCGTGGACA
TCGCCAAGAGTGAGATCTTCAATGGGGACGTGGAGTGGAAGTCCTGTGACCT
GGAGGAGGTGATGGTGGACGCCCTGGTCAGCAACAAGCCCGAGTTTGTGCGC
CTCTTTGTGGACAACGGCGCAGACGTGGCCGACTTCCTGACGTATGGGCGGCT
GCAGGAGCTCTACCGCTCCGTGTCACGCAAGAGCCTGCTCTTCGACCTGCTGC
AGCGGAAGCAGGAGGAGGCCCGGCTGACGCTGGCCGGCCTGGGCACCCAGC
AGGCCCGGGAGCCACCCGCGGGGCCACCGGCCTTCTCCCTGCACGAGGTCTC
CCGCGTACTCAAGGACTTCCTGCAGGACGCCTGCCGAGGCTTCTACCAGGAC
GGCCGGCCAGGGGACCGCAGGAGGGCGGAGAAGGGCCCGGCCAAGCGGCCC

ACGGGCCAGAAGTGGCTGCTGGACCTGAACCAGAAGAGCGAGAACCCCTGG
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ACTTCTGGGCCATGGGCCAGGAAGGTGTGGCAGCCGCACTGGCCGCCTGCAA
AATCCTCAAAGAGATGTCGCACCTGGAGACGGAGGCCGAGGCGGCCCGAGCC
ACGCGCGAGGCGAAATACGAGCGGCTGGCCCTTGACCTCTTCTCCGAGTGCT
ACAGCAACAGTGAGGCCCGCGCCTTCGCCCTGCTGGTGCGCCGGAACCGCTG
CTGGAGCAAGACCACCTGCCTGCACCTGGCCACCGAGGCTGACGCCAAGGCC
TTCTTTGCCCACGACGGCGTTCAGGCCTTCCTGACCAGGATCTGGTGGGGGA
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CTGTATGGCCTGCAGAGCCGGGTGGAGGAGCTGGTGGAGGCGCCGAGGGCTC
AGGGTGACCGAGGCCCACGTGCTGTCTTCCTGCTCACACGCTGGCGGAAATTC
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AGTTCACACTGTATGTGGGGGACAACTGGAACAAGTGTGACATGGTGGCCAT
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TCTTCAAGAAGGAGGCTGAGCACAAGCGGGAGCACCTGGAGAGAGACCTGC
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TCGGTGCTCGTGTCCTCCGTGGCTGACGTGCTGGCCCAGGGTGGCGGCCCCCG
GAGCTCTCAGCACTGTGGCGAGGGAAGCCAGCTGGTGGCTGCTGACCACAGA
GGTGGTTTAGATGGCTGGGAACAACCCGGGGCTGGCCAGCCTCCCTCGGACA
CATGAGCTGCTTGGCCTGCCACGTGTGGGGCCACCTCTCTTCAGTTGGCCACC
CTGCACGTTGTGCACTGACCTTTGCCGACCTCCAGCGGAACCCCCCAGGGGGC
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CCCAAAGGAACCGCTCCTTGTCCCTCCTGGCCTCCCCGGAGGCACAGCAGTGT
CATGGGGCTGTCTCCCCTGACAGGCACAACTCCCCGGGCAGAAAACGTGCCC
CACCGCATCCCTACCTGGAAACTGACCAGCCTGCACTGTGGAAAAGCTGGCC
CTGTGGCGTGACGGGGGAGCACCCCCATCCAGACTGCGAAGCTGCTCTGGGT
CTGCACCCACCCCTGCCCTGACTTGTGTTGCCTGACAAGAGACT

SEQ ID NO:8

Predicted human ltrpc6 amino acid sequence

translation from GI6716116

MQDVQGPRPGSPGDAEDRRELGLHRGEVNFGGSGKKRGKFVRVPSGVAPSVLF
DLLLAEWHLPAPNLVVSLVGEEQPFAMKSWLRDVLRKGLVKAAQSTGAWILTS
ALRVGLARHVGQAVRDHSLASTSTKVRVVAVGMASLGRVLHRRILEEAQEDFP
VHYPEDDGGSQGPLCSLDSNLSHFILVEPGPPGKGDGLTELRLRLEKHISEQRAGY
GGTGSIEIPVLCLLVNGDPNTLERISRAVEQAAPWLILVGSGGIADVLAALVNQPH
LLVPKVAEKQFKEKFPSKHFSWEDIVRWTKLLQNITSHQHLLTVYDFEQEGSEEL
DTVILKALVKACKSHSQEPQDYLDELKLAVAWDRVDIAKSEIFNGDVEWKSCDL
EEVMVDALVSNKPEFVRLFVDNGADVADFLTYGRLQELYRSVSRKSLLFDLLQR
KQEEARLTLAGLGTQQAREPPAGPPAFSLHEVSRVLKDFLQDACRGFYQDGRPG
DRRRAEKGPAKRPTGQKWLLDLNQKSENPWRDLFLWAVLQNRHEMATYFWA
MGQEGVAAALAACKILKEMSHLETEAEAARATREAKYERLALDLFSECYSNSEA
RAFALLVRRNRCWSKTTCLHLATEADAKAFFAHDGVQAFLTRIWWGDMAAGTP
ILRLLGAFLCPALVYTNLITFSEEAPLRTGLEDLQDLDSLDTEKSPLYGLQSRVEEL
VEAPRAQGDRGPRAVFLLTRWRKFWGAPVTVFLGNVVMYFAFLFLFTYVLLVD
FRPPPQGPSGPEVTLYFWVFTLVLEEIRQGFFTDEDTHLVKKFTLYVGDNWNKCD
MVAIFLFIVGVTCRMLPSAFEAGRTVLAMDFMVFTLRLIHIFAIHKQLGPKIIVVER
MMKDVFFFLFFLSVWLVAYGVTTQALLHPHDGRLEWIFRRVLYRPYLQIFGQIPL
DEIDEARVNCSTHPLLLEDSPSCPSLYANWLVILLLVTFLLVTNVLLMNLLIAMFS
YTFQVVQGNADMFWKFQRYNLIVEYHERPALAPPFILLSHLSLTLRRVFKKEAEH
KREHLERDLPDPLDQKVVTWETVQKENFLSKMEKRRRDSEGEVLRKTAHRVDFI
AKYLGGLREQEKRIKCLESQINYCSVLVSSVADVLAQGGGPRSSQHCGEGSQLV
AADHRGGLDGWEQPGAGQPPSDT

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/49808

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 33/53
US CL : 435/7.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/7.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00/45179 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 3 August 2000 (03.08.2000), see entire document.	1-15
A	WO 00/44929 A2 (THE REGENTS OFTHE UNIVERSITY OF CALIFORNIA) 3 August 2000 (03.08.2000), see entire document.	1-15
A	WO 00/40969 A1 (SIPPEL et al) 13 July 2000 (13.07.2000), see abstract.	1-15
A	OGURA et al, Bitter Taste Transduction of Denatonium in the Mudpuppy Necturus maculosus. The Journal of Neuroscience. 15 May 1997, Vol. 17, No. 10, pages 3580-3587, see entire document.	1-15
A	Database Medline on STN, AN 89362412, ROPER et al, "Distribution of ion channels on taste cells and its relationship to chemosensory transduction", Journal of Membrane Biology, July 1989, Vol. 109, No. 1, pages 29-39, see abstract.	1-15

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Date of the actual completion of the international search 30 April 2002 (30.04.2002)	Date of mailing of the international search report 20 MAY 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Richard Schnizer Felicia D. Roberts for Telephone No. 703-308-1235

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